BULLETIN OF MARINE SCIENCE
OF THE GULF AND CARIBBEAN

VOLUME 8 1958 NUMBER 3

STUDIES ON B VITAMINS IN RELATION TO PRODUCTIVITY OF THE BAHIA FOSFORESCENTE, PUERTO RICO

PAUL R. BURKHOLDER AND LILLIAN M. BURKHOLDER
Brooklyn Botanic Garden, Brooklyn, N. Y., and
University of Puerto Rico, Mayaguez, P. R.

ABSTRACT

The Bahia Fosforescente of Puerto Rico is luminescent because of a continuing bloom of dinoflagellates, a group including species known to be dependent for growth upon exogenous sources of B vitamins. Vitamin B_{12} in suspended solids of the bay ranged from 430 to 2930 mγ/gm. The ratio of B_{12} in suspended solids, to B_{12} in solution, was calculated to be about 2.5. Of the total B_{12} activity, determined with a mutant *E. coli*, from 7 to 23 per cent appeared to be cyanocobalamine, as indicated by *Ochromonas* assay. Averaged data from five selected stations in July of 1957, gave vitamin values in mγ/gm. of dried sediments as follows: B_{12}, 280; biotin, 7; B_1, 73. The calculated ratio of about 40:1:10 for these three vitamins is at variance with the estimated ratio of 1:1:800 for their proportions which would allow half maximum growth of vitamin-requiring microorganisms. It may be concluded that while B_{12} appears to be present in the bay sediments in relative excess, vitamin B_1 is probably a limiting factor for development of benthic populations. Data obtained for ten segments of a mud core suggest that B vitamins decrease with increasing depth and age of sediments. Large populations of both aerobic and anaerobic bacteria were found in various bottom sediments, consisting of fine ooze, shell and siliceous sands, and mangrove peat. Bacteria and actinomycetes in the drainage basin and bacteria in the marine water and sediments were observed to produce vitamin B_{12}. Although some B_{12} is undoubtedly contributed periodically from the watershed, synthesis of this vitamin occurs in the marine environment at significant physiological levels.

INTRODUCTION

Beautiful Bahia Fosforescente, situated near the town of La Parguera on the southwestern coast of Puerto Rico, is well known for its unusual intensity of luminescence at night. The estimated area of the bay is about 46 acres and the average depth approximately 3.5 meters. Mangrove forests occur around the margins of the bay (Fig. 1), except near the narrow outlet which connects with the ocean. Certain

fish (e.g. *Jenkinsia*) are abundant, oysters occur commonly along with sponges and coelenterates attached to mangrove roots, and plant and animal plankton appear to be generally thriving in the bay, in sharp contrast to less productive conditions in the clear, blue offshore

Figure 1. Aerial photograph of Bahia Fosforescente showing stations at which samples were obtained. Mangrove forests surround the bay everywhere except near the southern outlet, and salt flats occur to the north (at the top).

waters. Extensive commercial salt flats lie adjacent to the bay on the north. A large drainage area extends into the hill country beyond, from whence, during the rainy season, surface water carries silt and dissolved matter down into a canal which joins the middle portion of the bay. Two other drainage inlets occur near the salt flats on the northeast and northwest. Exchange of water between the bay and the ocean occurs through a shallow exit, approximately one hundred meters across. Since the average tide is only about one foot in this region, there appears to be comparatively little exchange of water through the narrow outlet between the bay and the ocean.

In view of the presence of numerous luminescent dinoflagellates and the high level of biological productivity in Bahia Fosforescente, it seemed important to make observations on the salinity, temperature, pH, plankton, bottom sediments, and suspended solids, along with determinations of important growth factors, such as vitamin B_{12}, biotin and thiamine. These vitamins are required by many dinoflagellates and other photosynthetic producers of primary organic matter of inshore marine environments (Sweeney, 1954; Lewin, 1954; Droop, 1955; Ford and Hutner, 1955).

It is a pleasure to acknowledge the very helpful cooperation of Dr. Juan A. Rivero, Director of the Institute of Marine Biology, University of Puerto Rico, Mayaguez, Puerto Rico, the encouragement given by Dr. R. E. Coker, and the technical assistance of Sr. Fermin Sagardia in concentrating cobalamine from sea water. Much of the work was done in the laboratory of the Institute of Marine Biology at La Parguera, Puerto Rico.

Methods

Bottom sediments were collected in a dredge and taken in screw capped bottles to the laboratory, where samples were spread in Petri dishes and air-dried rapidly. The dried sediments were stored in a desiccator until assays of vitamins could be made, within two to six weeks. Plankton was gathered by towing a number 20 silk net. Suspended solids, including plankton, bacteria, and detritus, were taken from water samples by filtration through weighed HA millipore filters. The water samples were obtained at the surface and near the bottom, using a special bacteriological sampler, holding a 500 ml glass-stoppered bottle. The solids retained on millipore filters were dried in an oven at 100°, cooled in a desiccator, and weighed. Determinations

of vitamin B_{12} activity were made with an *E. coli* mutant according to methods already described (Burkholder and Burkholder, 1956, 1956a) and with *Ochromonas malhamensis,* according to methods recommended by Hutner (1957). Biotin was assayed in dried sediments by using *Lactobacillus arabinosus.* (Barton-Wright, 1952) and thiamine activity was estimated by the *Phycomyces* method (Burkholder and McVeigh, 1940).

General Observations

The largest portion of the bay shows a gray-green to brown coloration that is in sharp contrast to the blue color of the clear ocean water outside. In the shallow upper arms of the bay the appearance of the water is dark brown, probably because of the presence of mangrove peat on the bottom. The central canal region has a gray-brown muddy appearance.

On February 6, 1957, samples of water were collected from nine stations ("A" series in Fig. 1) for various determinations. Temperature and specific gravity measurements were made on a line running from station 1A, in the canal, across the bay and out through the exit to the outside blue water at station 9A. Centigrade temperatures on February 6, 1957, varied as follows: Station 1A, 28.2°; 2A, 26.2°; 3A, 25.8°; 5A, 25.1°; 9A, 25.1°. According to records of R. E. Coker, made on March 22, 1957, the density of the surface water (calculated at 15°C) was 1.0276 at station 2 in the canal area, 1.0271 in the mid portion (station 5A), and 1.0265 outside the bay at station 9A. Surface temperatures on March 22 were recorded as follows: Station 1, 29.5°; 1A, 29.0°; 2A, 29.0°; 3A, 29.0°; 5A, 28.7°; 9A, 28.1°. These latter values show significant rise above the temperatures recorded on February 6, 1957.

Bottom sediments, water, and plankton samples were also obtained at some of the stations shown in Fig. 1 on February 5, and again on July 30 and 31, 1957. At the time when samplings were made at the end of July, the surface temperatures had risen to the following values: Station 2A, 30.5°; 3A, 29.2°; 7A, 29.8°; 9A, 28.5°. The difference in surface temperature of the main body of the bay and water outside the bay appears to be about one degree in the summer and less than this in the winter. Surface temperature of the bay and outside water rises in July to about 3.4 degrees above the values observed at the same stations in February. Transparency of the water,

indicated by Secchi disc readings made on July 31, 1957, was 3′-10″ at station 2A, and 11′-2″ at station 9A. The transparency of the outside water is always much greater than that in the bay, though detailed observations are not yet available for different months of the year.

Bottom Sediments. Microscopic examination of bottom sediments, obtained from stations indicated in Fig. 1, revealed the presence of several very different types of materials at various locations in the bay. It is obvious that the mangrove forest exerts an important influence upon the chemistry and biology of the bay and its sediments. Oyster shells occur abundantly on the bottom around the shallow margins of the bay, where they fall from the mangrove roots. Much organic detritus appears to accumulate in some bottom areas where mangrove leaves, stems and roots are found in various stages of decomposition, e.g., at stations 6, 11, 13, 15, 16, 17, 22, 23, 24, 25, 28, and 29. In July, there was a great abundance of mangrove peat on the bottom at station 12 (see Fig. 2).

Sand and clay mixed with fine organic detritus occurred in the upper portion of the canal at stations 1, 2, 3, and 4 (Fig. 2). Over much of the central area of the bay, (e.g., stations 6A, 8 and 9, and grading into other stations generally) fine ooze is present, apparently being derived from decomposing remains of plankton, and other plant and animal life. Finely ground rock and shell fragments (Fig. 2) occur as mixed sands near the mouth of the bay at such stations as 18, 19, 20, 30, 31 and 32. Very fine particles of silt are generally present at stations located in the main body of the bay between the central canal and narrow outlet.

This preliminary study of sediments permits us to point out that the origin and functions of the bottom deposits in relation to the fauna and flora, and the whole history of the bay, deserves intensive investigation. It appears that the large land drainage area above the bay contributes silt and dissolved substances in considerable amounts through a canal to the central portion of the bay during the rainy season, but in the dry season practically no runoff occurs from the land. The contribution of shells and shell fragments from oysters, is very considerable along the margins of the mangrove forest. This forest is green and vigorous, and seems to supply a very large amount of organic matter to the bay, in the same way that has been so well described for the mangroves of Florida by Davis (1940). Many kinds of attached marine algae and animals find homes on its fabulous root

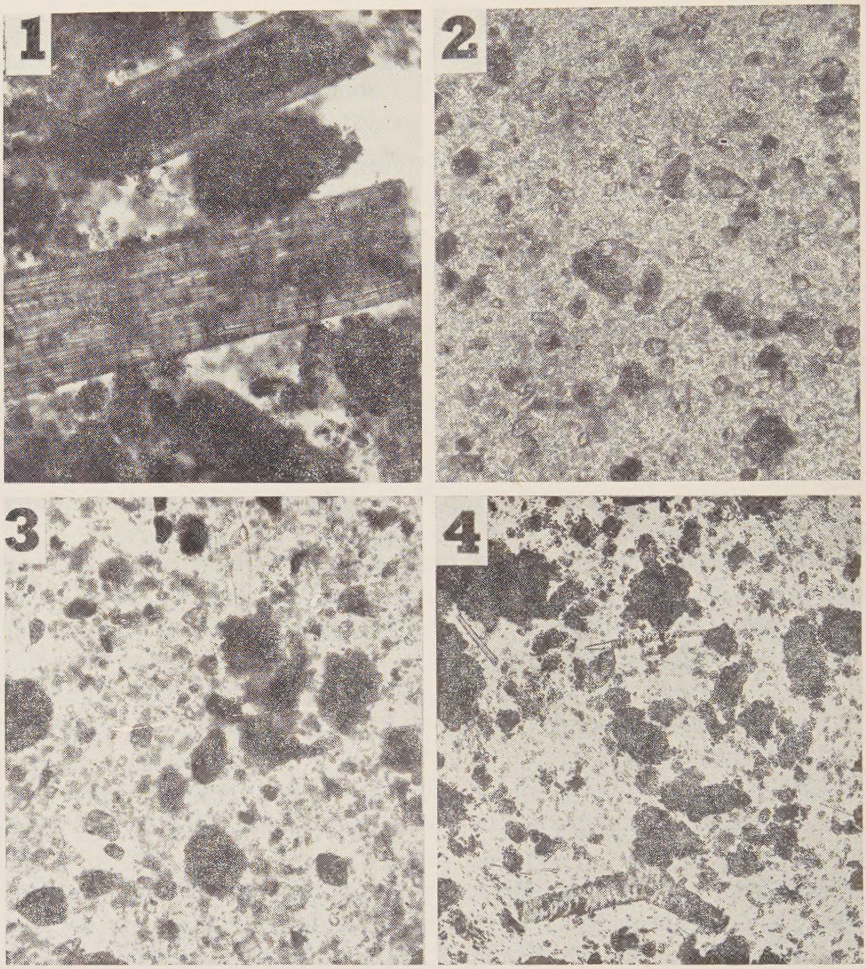


Figure 2. Photomicrographs (x 94) of sediments, collected in July 1957 from four different stations, illustrate the presence of diverse materials. Station 12 (photo 1) shows disintegrating peat composed of roots, stems and leaves of mangrove. Station 4 (photo 2) illustrates fine silt that appears to be contributed to the bay from watershed drainage entering through the central canal. Sediments of station 5A (photo 3) contain fine silt, particles of disintegrating mangrove, and fine organic ooze. At station 20 (photo 4), diatoms, and sponge spicules are mixed with coarse fragments of shells and mineral sands derived from nearby rocks.

system. The other main source of organic material, found in the bottom deposits, is derived from decomposing phyto- and zooplankton.

Because of the shallow nature of the bay, winds can stir the bottom mud and cause mixing of the waters periodically, thus supplying organic materials to the water mass which supports an abundance of plankton.

Plankton. Samples of plankton collected on February 5, 1957, with a No. 20 tow net indicate the presence of an abundant population of protozoa. The dominant photosynthetic organisms are dinoflagellates, including species of *Ceratium, Peridinium, Dinophysis, Pyrodinium,* and others (Fig. 3). *Pyrodinium bahamense* occurs abundantly, with lesser amounts of *Dinophysis caudata, Peridinium divergens, Ceratium furca, Peridinium oceanicum,* etc. (Margalef, 1957). A few diatoms (*Asterionella, Rhizosolenia,* and *Coscinodiscus,* are also present. Species of Rhizopoda are also fairly numerous, along with Ostracoda, Rotifera, etc. Several kinds of Copepoda constitute an abundant group

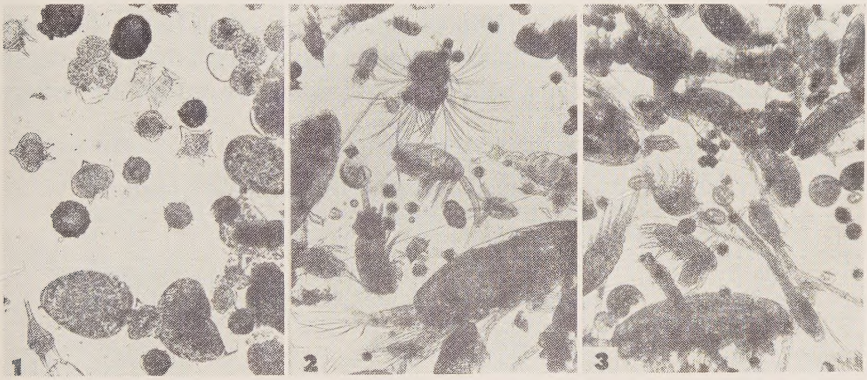


Figure 3. Photomicrographs of plankton communities present in Bahia Fosforescente, February 1957. Photo 1 (x 75) illustrates dinoflagellates that cause phosphorescence in the bay. Photos 2 and 3 (x 33) show copepods and other members of the zooplankton community. Many diatoms also appeared in the phytoplankton samples collected in July, 1957.

of small grazing animals in the zooplankton (Fig. 3). Among the various organisms which impart luminescence to the marine waters of Bahia Fosforescente, dinoflagellates are the most important group. Many of these are known to require B vitamins, e.g. vitamin B_{12}, thiamine and biotin (Provasoli and Pintner, 1953). Since these vitamins appear to be limiting growth of planktonic organisms in the ocean (Provasoli and Pintner, 1953; Sweeney, 1954; Droop, 1955, 1956), it is of interest to know about their quantitative occurrence in sus-

pended solids and sediments, and in solution in the waters of the bay.

Suspended Solids. Suspended matter of water samples, collected on February 5 and 6, and on July 30 and 31, 1957 from some of the stations shown in Fig. 1, was estimated by weighing the dried matter which was filtered on HA millipore filters (Millipore Filter Corp., Watertown 72, Mass., U.S.A.). Representative data, expressed as mg. per liter, are shown in Table 1. The suspended matter appears not to be uniformly distributed in the bay. In general, there is a tendency for higher values to be found in the water sampled from near the bottom, as compared with the surface. At station 6, samples were taken at a time (on February 5) when the prevailing wind had stirred up bottom sediments; the resulting high turbidity is indicated by the elevated dry weight values of 59.2 at the surface and 100.0 at the bottom. Suspended solids present in the clear water, at stations 8A and 9A, located several hundred yards outside the bay, are relatively low in both the surface and bottom samples. Microscopic examination of the suspended solids indicates that plankton accounts for most of the dry weight, except in special instances where wave action has stirred up the bottom

TABLE 1

DRY WEIGHTS OF SUSPENDED SOLIDS OBTAINED FROM A SERIES OF STATIONS IN BAHIA FOSFORESCENTE. VALUES ARE EXPRESSED AS MG. PER LITER OF WATER

Station	February Samples		July Samples	
	Surface	Bottom	Surface	Bottom
1A	8.7	8.7	—	—
2A	7.4	46.5	—	—
3A	6.7	35.3	—	—
4A	4.8	45.3	5.6	7.3
5A	7.4	10.7	—	—
6A	6.7	93.5	—	—
6 (muddy)	59.2	116.0	8.6	8.3
7A	6.1	45.2	—	—
8A	3.6	3.0	—	—
9A	1.3	1.5	2.0	—
10	13.3	79.7	—	—
12	—	—	8.9	10.9
13	13.7	16.7	—	—
14	—	—	6.3	9.9
16	11.2	35.5	—	—
22	23.8	27.0	—	—
23	—	—	7.9	7.9
25	10.2	24.5	—	—
26	14.3	25.7	11.9	28.4

mud. Comparison of the averaged data obtained for surface water at six stations in the winter and summer indicates the presence of somewhat more suspended matter in the winter (11.2 mg/l.) than in the summer (7.4 mg/l.).

Vitamins in Bottom Sediments. The values for vitamin B_{12} activity in bottom sediments were determined mainly in heated extracts of sulfite treated samples, employing the *E. coli* mutant assay. When samples were extracted by boiling in buffer solution without sulfite, the resulting data were considerably lower than the values found for sulfited samples. Typical results, reported in Table 2, indicate about three times as much B_{12} activity in the sulfited as compared with non-sulfited materials. This is in general agreement with our earlier work on sediments obtained from the Georgia sea island region (Burkholder and Burkholder, 1956).

When assays were performed with *Ochromonas malhamensis,* for the purpose of determining the specificity of B_{12} activity in these marine solids, some degree of sulfite inhibition of the assay organism

TABLE 2

Comparison of Values Obtained in Vitamin B_{12} Assays of Mud and Plankton Samples Extracted With and Without 0.1 per cent Sodium Sulfite. Data Are Expressed as mγ per Gram

Material	Without Sulfite	With Sulfite
Plankton from Bahia Fosforescente	300	1160
Plankton from Long Island Sound	140	600
Bahia Fosforescente Muds		
Station 14	250	740
Station 24	220	800
Station 28	300	940

was encountered. Dosage-response data, obtained with standard cobalamine in the absence of sulfite and in the presence of 0.1 mg of sodium sulfite per ml of growth medium, are shown in Table 3. It appears that differentiation between the fraction of B_{12} activity shown to be present by *Ochromonas,* as compared with the total indicated by *E. coli,* should rest upon data derived from plain buffer-extracted samples, even though the total assayable activity is lower. The data in Table 4, determined in the preferred manner on plankton and sediments, in order to avoid sulfite inhibition, suggests that only a minor portion of the total activity is actually cobalamine. Similar data have also been obtained for bottom sediments of Long Island Sound.

TABLE 3

DEPRESSING EFFECT OF SODIUM SULFITE UPON THE GROWTH RESPONSE OF *Ochromonas malhamensis* IN ASSAYS OF CYANOCOBALAMINE. GROWTH VALUES ARE EXPRESSED AS PER CENT LIGHT ABSORPTION IN A BAUSCH AND LOMB SPECTRONIC COLORIMETER

B_{12}, mγ/10 ml of medium	GROWTH	
	Without Sulfite	With Sulfite, 0.1 mg/10 ml
0	0	0
0.02	28	5
0.05	55	13
0.10	77	28
0.20	88	54
0.30	92	63
0.40	95	67
0.50	96	71

Results of *E. coli* assays for total B_{12} activity in bottom deposits at a number of stations in the bay are presented in Table 5. The B_{12} activity ranges from about 100 to approximately 2000 mγ of B_{12} per gram of dried bottom sediment. Highest values were found in sediments collected from around the margins of the bay, and particularly from the northern stations. This may be related to microbial activity in decomposing mangrove materials.

A core sample was obtained with a plastic tube at station 4 on July 31, 1957. The core, measuring about 83 cm in length x 7.7 cm in diameter, was cut into ten segments, the surface segment being 5 cm in length, and the other nine segments each about 8.7 cm in length. These were air dried, ground in a mortar, and then dried in a

TABLE 4

VITAMIN B_{12} FOUND IN MARINE SOLIDS ASSAYED WITH *E. coli* AND WITH *Ochromonas malhamensis*. SAMPLES WERE EXTRACTED IN CITRATE BUFFER WITHOUT SULFITE. VALUES ARE EXPRESSED AS mγ PER GRAM OF DRY SOLIDS, COLLECTED IN FEBRUARY, 1957

Samples	A *Ochromonas* assay	B *E. coli* assay	$\frac{A}{B} \cdot 100$
Suspended Solids, Bahia Fosforescente	40	300	13.3
Suspended Solids, Long Island Sound	10	140	7.1
14 Mud, Bahia Fosforescente	18	250	7.3
24 Mud, Bahia Fosforescente	50	220	23.0
28 Mud, Bahia Fosforescente	55	300	18.3

desiccator over "drierite." A small portion of each core segment was assayed for cobalamine, biotin, and thiamine activity. The results, shown in Table 6, indicate that the vitamin B_{12} concentration steadily decreases with increasing depth below the surface of the mud. The appearance of this core changed from gray-brown fine silt and ooze at the surface, to brown-black layers below, and abundant shell fragments mixed with the mud in segments 9 and 10 at the bottom of the core.

TABLE 5

VITAMIN B_{12} OF BOTTOM DEPOSITS, PREPARED IN SULFITE BUFFER, AND ASSAYED WITH *E. coli*. DATA WERE CALCULATED AS mγ PER GRAM OF DRIED SEDIMENTS. THE VARIOUS STATIONS (FIG. 1) ARE HERE GROUPED ACCORDING TO FOUR DIFFERENT RANGES OF B_{12} CONCENTRATION

Range of B_{12}	100-300	301-500	501-1000	1001-2000
February Stations	1	4	14	
	2	6	17	
	3	7	22	
	5	9	24	
	8	10	26	
	12	11	27	
	13	25	28	
	15	29		
	16	30		
	18			
	19			
	20			
	21			
	23			
	31			
	32			
July Stations	3	4	23	5
	8	20		14
	11			15
	13			

Biotin was assayed in seven portions of the core. The samples were prepared by digestion with 3N H_2SO_4 in an autoclave, cooled, neutralized and filtered prior to assaying with *Lactobacillus arabinosus*. The resulting data, presented in Table 6, show relatively high values in segments 1 and 6, and the lowest in segment 10 at the bottom.

Other biotin determinations, made on surface sediments at four additional stations, showed the following values expressed in mγ/gm of dried mud: 8.7, 8.0, 8.0, and 11.0 at stations 8, 11, 13 and 14, respectively. The ratio of vitamin B_{12} to biotin averaged 32:1 in ten

TABLE 6

DISTRIBUTION OF VITAMIN B_{12}, BIOTIN, AND THIAMINE IN A CORE SAMPLE FROM STATION 4. ALL VALUES ARE EXPRESSED AS mγ OF VITAMIN PER GRAM OF DRIED MATERIAL

Core Segments No.	Depth	Vitamin B_{12}	Biotin	Thiamine
1	0 - 5.0 cm (top)	200	5.0	63
2	5.0 - 13.7 cm	162	3.2	40
3	13.7 - 22.4 cm	100	3.2	35
4	22.4 - 31.1 cm	110	—	—
5	31.1 - 39.8 cm	110	—	—
6	39.8 - 48.5 cm	100	5.0	10
7	48.5 - 57.2 cm	84	4.0	7
8	57.2 - 65.9 cm	66	3.2	3
9	65.9 - 74.6 cm	30	—	—
10	74.6 - 83.3 cm (bottom)	30	1.2	0

different samples of mud.

Thiamine was found to be present in such biologically low amounts that the assays proved difficult to make. Several trials were made with pepsin digested mud samples, prepared for assays with *Lactobacillus fermenti*. The levels of thiamine were found to be too low to stimulate growth of the assay bacterium. Some success was attained by using a *Phycomyces* assay. Determinations were made by using 2.0 gm of crude dry sediment added directly to 25 ml of assay medium contained in 125 ml Erlenmeyer flasks. After sterilization by autoclaving, the flasks were inoculated with one drop of *Phycomyces* spore suspension. Growth was allowed to proceed for one week at 25° C, after which time the mycelium was dried and weighed. Calculations of thiamine present in the mud samples were made in the usual way (Burkholder and McVeigh, 1940).

The results obtained with *Phycomyces* should be viewed as "vitamin B_1 activity," because of the response of the assay fungus to thiamine and also to the pyrimidine plus thiazole components of the vitamin molecule. Thiamine activity data for seven segments of a core sample are presented in Table 6. Vitamin B_1 activity is highest in the top segment of mud and decreases steadily downward to 0 at the bottom of the core. Vitamin B_1 values, determined in sediments from four other locations, were 10, 40, 90, and 195 mγ per gram of dry matter, at stations 13, 11, 14 and 8, respectively.

It is important to consider the possible biological significance of the levels of these vitamins for growth of organisms in the sediments and

bottom waters of the bay. The vitamin data for five different samples (from stations 4, 8, 11, 13 and 14) show an average content (in mγ/gm of dry material) of 280 for B_{12}, 7 for biotin, and 73 for B_1, or a ratio of roughly 40:1:10. For half maximum growth of microorganisms, using the responses of the three assay organisms employed in this work, the calculated ratios are approximately 1:1:800. If organisms growing in marine mud require these vitamins in about the same ratio, then it is apparent that the proportions of vitamin occurrence and vitamin requirement are not similar in these sediments. For half maximum growth of deficient microbes per gram of aqueous medium, about 0.02 mγ of B_{12} or of biotin would suffice, but approximately 16 mγ of B_1 would be needed. If we divide the amount of vitamin required for half maximum growth into the amounts actually found, then the following relations appear: for B_{12}, 280 0.02; for biotin, 7/0.02; and for B_1, 73 16. These quotients suggest that B_{12} is in excess of half maximum requirements in mud by 14,000 fold, biotin by 350, and B_1 by about 4.5 times. Apparently one gram of mud could supply sufficient B_{12} for 14 liters of sea water medium, to allow half maximum growth of microorganisms that require this vitamin.

Vitamin B_{12} of Suspended Solids. Assays of vitamin B_{12} in suspended solids, collected on millipore filters, were made with the *E. coli* mutant method. The resulting data, for stations sampled in February (Fig. 1), are presented in Table 7. In general, there seems to be a fairly high concentration of B_{12} in these samples of suspended matter, the values ranging from 430 to 2930 mγ of B_{12} per gram of dried solids. These data have been calculated, also, on the basis of vitamin B_{12} of suspended solids contained in one liter of water. The data for total amount of B_{12} carried on the solids in a liter show consistently higher values at the bottom, as compared with the surface, for all samples taken inside the bay. The B_{12} of normal surface samples in the bay ranges from 3.3 to 40.0 mγ per liter, whereas at the bottom the range is from 17.5 to 86.7. At the muddy water station (No. 6), assays of the surface showed 100.0, and the bottom, 146.7 mγ per liter. Outside the bay, at stations 8 and 9, total values are low at both surface and bottom, the range being 2.5 to 3.3 mγ per liter. Though the concentration of B_{12} activity (in mγ/gm) is fairly high in suspended solids of the clear water, the total amount of B_{12} expressed as mγ/liter is much less than that present in bay water.

Analyses of B_{12} in the suspended solids of six stations in the bay were made in July, 1957. The average value for the surface was 824 and at the bottom 995 mγ/gm of suspended matter. The same analyses, expressed in mγ/ liter of water, gave averages of 6.3 for the surface and 13.4 for the bottom samples. A sample of surface water, taken from station 9A outside the bay, assayed 600 mγ/gm and 1.2 mγ/liter. It appears that the total vitamin B_{12} of suspended matter is much less in July than in February, and at both times less outside the bay than inside.

Dissolved Vitamin B_{12}. For the determination of dissolved vitamin B_{12}, samples were collected in May 1957, at stations 11 and 28 in the bay, and at station 9A outside the bay. The water was filtered through millipore filters in order to remove the suspended matter. Then the vitamin B_{12} was concentrated 10 fold by extraction with phenol according to the method of Cowey (1956), modified by use of sulfite instead of cyanide during extraction. Assays were performed with *E. coli,* after removing all traces of phenol by thorough washing with ether. Values of 3.0 and 3.5 mγ/liter were obtained for the bay stations 11 and 28, and 1.3 mγ/liter for station 9A outside the bay.

Vitamin B_{12} in the Drainage Basin. Soils were collected from many sites in the grassy fields of the drainage basin lying to the north

TABLE 7

VITAMIN B_{12} IN SUSPENDED SOLIDS ASSAYED WITH *E. coli.*
DATA ARE GIVEN IN mγ PER GRAM OF DRIED SOLIDS AND PER LITER OF WATER

Station	Depth in feet	B_{12} per gram		B_{12} per liter	
		Surface	Bottom	Surface	Bottom
1A	1	847	847	7.3	7.3
2A	4	932	430	7.2	20.0
3A	4	1313	693	8.8	17.5
4A	12	1041	882	5.0	40.0
6A	12	1325	780	8.8	73.3
6 (muddy)	12	1690	1264	100.0	146.7
7A	12	891	588	5.3	36.7
8A	14	909	833	3.3	2.5
9A	12	1875	1667	2.5	2.5
10	13	1100	1099	14.7	86.7
13	6	2930	2400	40.0	40.0
16	8	1071	—	12.0	—
22	3	625	865	15.0	23.3
25	5	1010	940	10.3	23.3
26	5	954	910	13.7	23.3

of Bahia Fosforescente, and also along the muddy banks of the canal which joins the bay. These samples were pooled into five different lots for vitamin B_{12} analysis. The data suggest that the soils and mud above the bay are low in vitamin B_{12} content, as compared with the bottom sediments and suspended solids in the bay. Three pooled samples of soil yielded values of 36, 50, and 80 mγ per gram of dried material. Samples of sandy loam near the canal entrance to the bay averaged only 11, and mud from the bank of the canal itself averaged 60 mγ per gram. It is probable that vitamin B_{12} may be produced mainly during periods favorable for development of bacteria and actinomycetes, which were found to be present in good numbers in the soils of this drainage area. During dry seasons, metabolic activities of the microorganisms is probably at a low level in this semi-arid region. It seems probable, nevertheless, that relatively large amounts of vitamin B_{12} may be washed down from the land into the bay during heavy rains.

The occurrence of "red tides" caused by the growth of planktonic dinoflagellates has been observed by Dr. J. Rivero in the shallow coastal waters near La Parguera. Aerial photographs often show red patches of water on the lee side of mangrove areas. It appears that conditions in or near mangrove swamps may be favorable for development of flagellate blooms. It is not clear whether algal growth occurs in the mangrove forest, followed by displacement of the red water by prevailing winds, or whether enriched waters are displaced from their origin and favor growth on the leeward side of islands and bays. It is known that mineral nutrients and B vitamins are required for the rapid growth of dinoflagellates that occur in red water, and much work has been done on the cause of red tides, because of the occasional association with extensive mortality of fish.

Mud and water were collected from beneath red tides at La Parguera by Dr. J. Rivero for analysis of vitamin B_{12}. The different samples were allowed to stand in glass bottles in the laboratory until bacterial growth became obvious; then tests were made for B_{12} in the sea water solution above the mud, using a plate assay method. Some showed B_{12} values of about 100 mγ per ml (see Fig. 4). Other samples of mud and scrapings from mangrove roots assayed about 30 mγ of B_{12} per ml of supernatant sea water. All of these samples of mud and water, collected from the environments of red tide, exhibited vitamin B_{12} activity in excess of the growth requirement of dinoflagel-

tates. Since the levels of this vitamin show higher concentration in the marine sediments than in the soils of the watershed, it does not seem necessary to derive B_{12} from the land drainage in order to explain the presence of adequate supplies for growth of red tide organisms.

Vitamin B_{12} Production by Microorganisms. Samples of mud obtained from twelve stations were examined for the presence of aerobic and anaerobic bacteria. Some of the stations were selected because of the presence of H_2S in the bottom sediments (e.g., stations *13* and *22*). Numerous aerobes and fewer anaerobes were found in the samples

Figure 4. A Petri dish shows microbiological assay of marine materials for vitamin B_{12} using a mutant strain of *E. coli* seeded in nutrient agar lacking B_{12}. Growth zones of bacteria requiring B_{12} are seen to occur only near pads of filter paper that have been impregnated with mud or water possessing vitamin activity. Numbers 1, 2, and 7 show large growth zones resulting from applications of sediments and sea water obtained from a region of red tide; 3 and 4 exhibit responses to scrapings from mangrove roots; pad 5 contains mud collected under a red bloom; 6 illustrates that B_{12} is present in material that came from a bed of *Thalassia* under a red tide.

TABLE 8

QUANTITATIVE OCCURRENCE OF AEROBIC AND ANAEROBIC BACTERIA IN BOTTOM DEPOSITS OF BAHIA FOSFORESCENTE. DATA ARE EXPRESSED IN MILLIONS PER ML

Station	Aerobic	Anaerobic
10	55	—
32	65	10
25	70	28
19	137	9
31	171	—
9	229	133
3	236	—
23	274	—
11	455	346
22	885	380
13	995	—
28	2440	—

studied. The numbers of aerobic organisms in the bottom mud were estimated to be in the range of 55 to 2400 millions per gram of dried mud. The quantitative occurrence of bacteria in mud samples obtained from 12 different stations is presented in Table 8.

Production of vitamin B_{12} by pure cultures of marine bacteria isolated from mud taken from Bahia Fosforescente was studied in sea water enriched with the following compounds, stated in grams per liter: K_2HPO_4, 0.02; $MgSO_4 \cdot 7H_2O$, 0.10; $(NH_4)_2SO_4$, 1.0; sodium citrate $\cdot 3H_2O$, 0.50; glucose, 10.0; asparagine, 4; and 0.05 of each of the amino acids, arginine, glycine, glutamic acid, histidine, proline, and tryptophan. Cobalt chloride was added to provide 2 p.p.m. of cobalt. Pure cultures were allowed to grow for three days in this medium. Tests for vitamin B_{12} were made with paper pads impregnated with the different beers and placed upon special assay nutrient agar plates seeded with the mutant *E. coli*. Among 60 isolates tested, 12 showed B_{12} activity in the range of 0.5 to 10.0 mγ per ml of beer. These values come in the range of production recently reported for marine bacteria by Starr, Jones and Martinez (1957).

From soils collected in the watershed above the bay, 105 cultures of bacteria and actinomycetes were isolated for tests of their capacity to produce B_{12}. These cultures were grown in the same medium mentioned above, except that phosphates were increased to 7.0 gm per liter and distilled water was used in place of sea water. The beers were assayed with the agar plate method. Among 60 bacterial isolates, 22

produced B_{12} in the range of 0.3 mγ to somewhat more than 100 mγ per ml of broth. Among 45 actinomycetes, 42 produced B_{12} in the range of 1.0 mγ to 100 mγ ml. It is obvious that numerous organisms from the soils of the watershed are able to produce vitamin B_{12} in appreciable amounts. It is assumed that these bacteria and actinomycetes contribute to the store of B_{12} found in their native habitat, and that some of this may be washed down into the sea during heavy rains.

Discussion

Bahia Fosforescente is an excellent example of a tropical inshore environment where primary productivity comes from a dinoflagellate phytoplankton and mangrove forest. Its mineral nutrient level, suitable for supporting a rich fauna and flora, is apparently derived from land drainage and maintained by circumstances related to its partial isolation from the open sea. If the suspended solids, comprised mainly of plankton, may be taken as an index to productivity, then it becomes apparent that the bay is about ten times as rich as the sea waters outside the bay.

It is of some interest to compare the mangrove-planktonic Bahia Fosforescente with other inshore types of environment, such as marshes of the Georgia sea islands and the planktonic waters of Long Island Sound. Primary productivity in the Georgia coastal waters is derived from a mixed population of flagellates and diatoms, and abundant *Spartina* in the marshes. A periodic diatom phytoplankton and benthonic algae contribute the chief primary organic matter in Long Island Sound. We have compared the weight of suspended solids collected from the surface of Bahia Fosforescente on February 6, 1957, with the solids taken from a station near New Haven harbor in Long Island Sound on March 6, 1957, during a spring bloom of diatoms. An average of six surface samples taken from the bay yielded a value of 14.4 mg per liter, and four samples from the sound gave a comparable figure of 16.8 mg per liter. The suspended solids of the waters around Sapelo Island, Georgia, collected during a plankton bloom on November 28, 1955, ranged from 22 to 159 mg per liter, depending upon whether the samples were taken in sparse or dense zones of growth. If one considers that the production of phytoplankton in temperate regions is periodic, it appears possible that a tropical bay, such as that under consideration here, may produce more steadily

throughout the year and yield a very high total amount of organic matter. The ratio of grass phytoplankton to the grazing zooplankton, periodic fluctuation in kind and amount of suspended matter, chlorophyll as an index to CO_2 reduction, properties of sediments, role of bacterial transformations, the seasonal changes of physical and chemical factors in the bay, provide many problems which deserve consideration in the future.

Because of the importance of vitamins in the life of marine organisms, it becomes necessary to develop methods and gather factual information concerning the occurrence of these growth factors in order to develop a better understanding of marine ecology and productivity. Vitamin B_{12}, thiamine, and biotin (Provasoli and Pintner, 1953) are essential for growth of many photosynthetic species of marine phytoplankton. Emphasis in the present paper is given to studies of the vitamin B_{12} complex in particulate matter in suspension and in bottom deposits. Perhaps of even greater importance is the dissolved vitamin B_{12}, though accurate determination of this fraction apparently requires special concentrating techniques (Cowey, 1956).

With comparable methods of collecting and assaying marine suspended solids and muds, in three inshore regions referred to above, it is possible to make certain preliminary comparisons of conditions which existed at stated times. Vitamin B_{12} in bottom deposits of the bay ranged from about 100 to 2000 mγ per gram of dried matter. Six samples of intertidal mud taken from marshes of Sapelo Island, Georgia, ranged from 520 to 870, although other determinations ran as low as 190 mγ/gm. Seven samples of bottom mud collected during the summer of 1957 from Long Island Sound, showed a range of 56 to 840 mγ per gram of dried mud. The concentration of vitamin B_{12} in suspended solids of Bahia Fosforescente, reaching as high as 2930 mγ/gm, is generally higher than in comparable samples taken from the marine environment of the Georgia sea island region (Burkholder and Burkholder, 1956), where the highest value was 736 mγ/gm of suspended solids. Ten surface water samples taken in October off the coast of Georgia, average 226 mγ/gm of solids, and ten surface samples taken in February from Bahia Fosforescente averaged 1325 mγ/gm. Assays of plankton taken in a No. 20 silk net, gave an average figure of 600 mγ per gram for Long Island Sound, and a value of 1160 for Bahia Fosforescente. It is apparent that the suspended solids and plankton in the tropical bay are relatively rich in vitamin B_{12}, but

whether this comes about chiefly as a result of synthesis *in situ* or by absorption from solution in the water remains to be demonstrated. The few assays of vitamin B_{12} present in solution and that contained in suspended solids of surface waters in Bahia Fosforescente during the summer of 1957 gave an average ratio for

$$\frac{B_{12} \text{ in suspension}}{B_{12} \text{ in solution}} = \frac{8.20}{3.25} = 2.52.$$

It would be of interest to know whether some similar ratio would be found in further studies on the problem.

A recent study by Cowey (1956) of vitamin B_{12} in marine plankton, and that dissolved in ocean waters, is important both for its methods and results. Zooplankton was found to contain more vitamin B_{12} than phytoplankton, but it is not known whether the richness of zooplankton derives from rapid grazing or from a bacterial flora in the animals. Assays of dissolved B_{12}, after extraction from the sea water and concentration with phenol, showed considerable seasonal variations in the North Sea. Since Cowey's data for marine organisms were determined without sulfite extraction and expressed on a wet-weight basis, it is difficult to make accurate comparisons of our data with results which he obtained in northern European waters. Differential assays, performed with *Lactobacillus* and *Ochromonas,* led Cowey to conclude that analogs in addition to cyanocobalamine are present in the sea. Similar conclusions were reached in the paper by Starr, Jones and Martinez (1957). The few data presented in our Table 4 permit a calculation of per cent true vitamin $B_{12} = \frac{\textit{Ochromonas} \text{ activity}}{\textit{E. coli} \text{ activity}} \times 100.$ The range of calculated values is from 7.1 to 23.0. Such data suggest a tentative conclusion that although a significant fraction of the total vitamin B_{12} complex in marine solids is actually vitamin B_{12} of a type active for *Ochromonas* and higher animals, the largest part of the cobalamine complex in the sea may be represented by other forms than cyanocobalamine. It still appears reasonable to assay total B_{12} activity for marine microorganisms with a method that employs *E. coli,* or some similar organism, possessing a broad capacity of response to different forms of B_{12}.

Although our data are meager with regard to distribution of B vitamins in core samples, the results presented in Table 6 indicate decline of the values for B_{12}, biotin and thiamine with increasing depth

of the sediments. It appears that at least some members of the B complex of vitamins may not be preserved as well as certain carotene pigments in sediments (Vallentyne, 1954; Fox and Anderson, 1941). The presence of physiologically low amounts of thiamine indicates the need for improved methods of assay.

More information concerning the distribution of vitamins, amino acids, carbohydrates, and other growth substances in the ocean is greatly needed for a proper understanding of marine ecology. New techniques of extracting growth factors out of sea water, and direct assays with micronutrient-requiring marine microorganisms, or with biochemical mutants adapted to the salinity of the sea, would open the way to an understanding of the complex growth factor interactions in productivity.

Summary

Quantitative occurrence of the B vitamins, cobalamine, biotin, and thiamine, was studied in suspended solids and muds collected from the Bahia Fosforescente during February and July, 1957. In contrast to the clear blue waters off shore, where very little suspended matter is present, a moderately abundant standing crop of plankton is present through the year in this inclosed tropical bay. Striking luminescence is ever present at night because of the continuing bloom of dinoflagellates.

The surface temperatures in February and July at a typical station in the bay were found to be 25.8° and 29.2° respectively, and for the same times outside the bay, the surface temperatures were 25.1 and 28.5°. The difference in surface temperature inside and outside the bay is usually about one degree or less, and in July, the temperature of the bay and outside water rises to about 3.4 degrees higher than that in February.

Vitamin B_{12} in suspended solids of the bay was relatively high, ranging in different samples from 430 to 2930 mγ/gm. The ratio of B_{12} in suspended solids/ B_{12} in solution was found to be about 2.5 in the bay. Of the total B_{12} activity, determined with a mutant *E. coli,* from 7 to 23 per cent appeared to be cyanocobalamine, as indicated by *Ochromonas* assay.

Averaged data for sediments obtained from five selected bay stations in July gave vitamin values (in mγ/gm of dried mud) of 280 for B_{12}, 7 for biotin, and 73 for B_1, or a ratio of about 40:1:10. For growth of vitamin requiring oranisms present in these muds, it appears that the

calculated ratios for half maximum growth would be more like 1:1:800 expressed in weight units of B_{12}:biotin:B_1. It may be concluded, therefore, that B_{12} is in relative excess, while thiamine may be a limiting factor in the development of benthic populations. Data obtained for ten segments of a mud core suggest that B vitamins may decrease steadily with increasing depth and age of the sediments.

Assays of numerous pooled samples of soils, collected from the drainage basin of the bay in February, yielded values for B_{12} in the range of 36 to 80 mγ/gm of dried material. Comparable B_{12} assays of bottom mud collected from the bay in February gave values ranging from about 100 to nearly 1000 mγ/gm. Determinations of B_{12} in solution in water and mud mixtures collected from regions of red tide yielded results in the range of 30 to 100 mγ per ml.

Quantitative determinations of aerobic bacteria present in 12 different mud samples from the bay, gave estimates in the range of 55 to 2400 millions per gram of dried mud. Anaerobic bacteria in these same samples ranged from 9 to 380 millions per gram. Among 60 isolates of marine bacteria grown in enriched sea water medium, 12 showed B_{12} activity up to 10 mγ per ml of fluid. Among 105 cultures of bacteria and actinomycetes isolated from soils of the watershed, 22 of 60 bacteria, and 42 of 45 actinomycetes formed B_{12} in varying amounts up to 100 mγ/ml of nutrient broth. It is concluded that although some B_{12} is undoubtedly contributed periodically from the watershed, synthesis of this vitamin probably occurs in the bay at significant physiological levels.

LITERATURE CITED

Barton-Wright, E.
1952. The microbiological assay of the vitamin B-complex and amino acids. Pitman Publ. Corp., New York. 179 pp.

Burkholder, P. R.
1951. Determination of vitamin B_{12} with a mutant strain of *Escherichia coli*. Science, *114:* 459-460.

Burkholder, P. R. and L. M. Burkholder
1956. Microbiological assay of vitamin B_{12} in marine solids. Science, *123:* 1071-1073.
1956a. Vitamin B_{12} in suspended solids and marsh muds collected along the coast of Georgia. Limnol. and Oceanogr., *1:* 202-208.

Burkholder, P. R. and Ilda McVeigh
1940. Studies on thiamine in green plants with the Phycomyces assay method. Amer. J. Bot., *27:* 853-861.

Burton, M. O. and A. G. Lochhead
1951. Studies on the production of vitamin B_{12}—active substance by microorganisms. Can. J. Bot., *29:* 352-359.

Cowey, C. B.
1956. A preliminary investigation of the variation of vitamin B_{12} in oceanic and coastal waters. J. Mar. Biol. Assoc. U. K., *35:* 609-620.

Davis, J. H.
1940. The ecology and geologic role of mangroves in Florida. Pap. Tortugas Laboratory, *32:* 305-412. 12 plates.

Droop, M. R.
1955. A pelagic marine diatom requiring cobalamine. J. Mar. Biol. Assoc. U. K., *34:* 229-231.

Ford, J. E. and S. H. Hutner
1955. Role of vitamin B_{12} in metabolism of microorganisms. Vitamins and Hormones, *13:* 102-133.

Fox, D. L. and L. J. Anderson
1941. Pigments from marine muds. Proc. Nat. Acad. Sci., *27:* 333-337.

Hutner, S. H.
1957. Personal communication.

Lewin, R. A.
1954. A marine Stichococcus sp. which requires vitamin B_{12} (cobalamine). J. Gen. Microbiol., *10:* 93-96.

Margalef, Ramón
1957. Fitoplancton de la costas de Puerto Rico. Invest. Pesq., Barcelona., *6:* 39-52.

Provasoli, L. and J. J. Pintner
1953. Ecological implications of in vitro nutritional requirements of algal flagellates. Ann. N. Y. Acad. Sci., *56:* 839-851.

Starr, T. J., N. E. Jones and D. Martinez
1957. The production of vitamin B_{12} active substances by marine bacteria. Limnol. and Oceanogr., *2:* 114-119.

Sweeney, B. M.
1954. Gymnodinium splendens, a marine dinoflagellate requiring vitamin B_{12}. Amer. J. Bot., *41:* 821-824.

Vallentyne, J. R.
1954. Biochemical limnology. Science, *119:* 605-606.

TWO NEW PLEXAURID GORGONIANS FROM THE BAHAMA ISLANDS[1]

FREDERICK M. BAYER
U. S. National Museum
AND
ELISABETH DEICHMANN
Museum of Comparative Zoölogy

ABSTRACT

Two species of the reef dwelling gorgonacean family Plexauridae are described as new. Both were recovered from a habitat unusually deep for genera concerned (36 fathoms) and show modification toward the slender, delicate colonial form characteristic of deep-water genera. *Plexaura nina* bears a strong resemblance to *P. homomalla* (Esper), the generic type, but differs both in growth form and in spicular details. *Eunicea pinta* belongs to the group of species related to *Eunicea mammosa* Lamouroux, also the type of its genus, but lacks protruding calicles and has atypically strong anthocodial armature, as well as differing in growth form and details of spiculation.

INTRODUCTION

Amongst the shallow-water gorgonians of the West Indian region, perhaps none are more conspicuous and characteristic of the reef fauna than the plexaurids. It is accordingly of no little interest that a tangle haul made at a depth of 36 fathoms in the Tongue of the Ocean, Great Bahama Bank, by the U. S. Fish Commission steamer ALBATROSS during 1886 contains representatives of two typically reef dwelling plexaurid genera. Both demonstrate the effect that a deep, quiet habitat has upon a colonial form adapted for life in the turbulent waters of the reef. Instead of the densely arborescent, thick-branched growth form usually met with in shallow water on the reefs, these two species are sparingly branched, with exceptionally long, slender, and flexible terminal branches. In both, the anthocodial armature is unusually strong for plexaurids, which commonly have only a weak crown or merely a few tiny, tentacular rods, sometimes none at all. In consequence, the anthocodiae of both species tend to remain exsert in preservation to a much greater degree than is usual for reef dwelling plexaurids.

Similar modifications can be seen in the deeper-water representatives of the genus *Muricea,* as well as among those of several gorgoniid genera. The colonies assume a spindly and leggy aspect while retaining the customary pattern of ramification; the anthocodial armature becomes unusually strong; and the spicules of the rind tend to become

[1]Published with the permission of the Secretary of the Smithsonian Institution.

more aculeate. Thus, the effects of the physical environment seem to elicit similar responses in members of various holaxonian genera in at least two families.

One of the species obtained by the ALBATROSS bears obvious resemblance to the shallow-water group of *Plexaura homomalla* (Esper) and therefore is referable to the genus *Plexaura,* and the other shares many features in common with the reef-dwelling group of *Eunicea mammosa* Lamouroux, but the generic distinction, unsatisfactory at best, is even more obscure in these two deeper water forms. The type species of the genus *Eunicea, E. mammosa,* has its surface spicules in the form of aculeate torches, and contains long, slender, bright purple, spinose spindles in the axial sheath layer, with capstans appearing only in the larger branches near the base of the colony. Contrastingly, the axial sheath of *Plexaura homomalla,* type species of *Plexaura,* contains abundant capstans or spheroidal bodies all the way to the branch tips, thus providing one of the few distinguishing features that seems to hold good for separation of these genera. Unfortunately, both of the species now under consideration have capstans in the axial sheath all the way up to the branch tips, although only one of them has torches of the *Eunicea* type. In the latter, the capstans are found to be rather acutely pointed and mostly sculptured with only conical spines rather than with complex tubercles and so correspond with those found in some species of *Eunicea.* Thus it still seems justifiable to retain the distinction between *Plexaura* and *Eunicea,* and to assign one of these new species to the former and the other to the latter.

Genus *Plexaura* Lamouroux

Plexaura Lamouroux, 1812, p. 187. (Type species, *Gorgonia homomalla* Esper, by subsequent designation: Verrill, 1912, p. 382.)
Plexaura Moser, 1921, p. 110.
Plexaura Kükenthal, 1924, p. 111.
Plexaura Stiasny, 1935, p. 44.

Diagnosis.—Plexaurids with cortical spiculation divided into three layers: outer layer clubs (chiefly leaf-clubs), often with some unilaterally foliate capstans or spindles; middle layer predominantly spindles of large (up to 2 mm) or moderate size; inner layer (=axial sheath) with capstans of deep reddish purple color predominating. Anthocodiae strongly armed with spindles forming a crown with 8 points, flattened rods in the backs of the tentacles, and sometimes a collaret.

Remarks.—Of the nineteen species attributed to this genus by Küken-

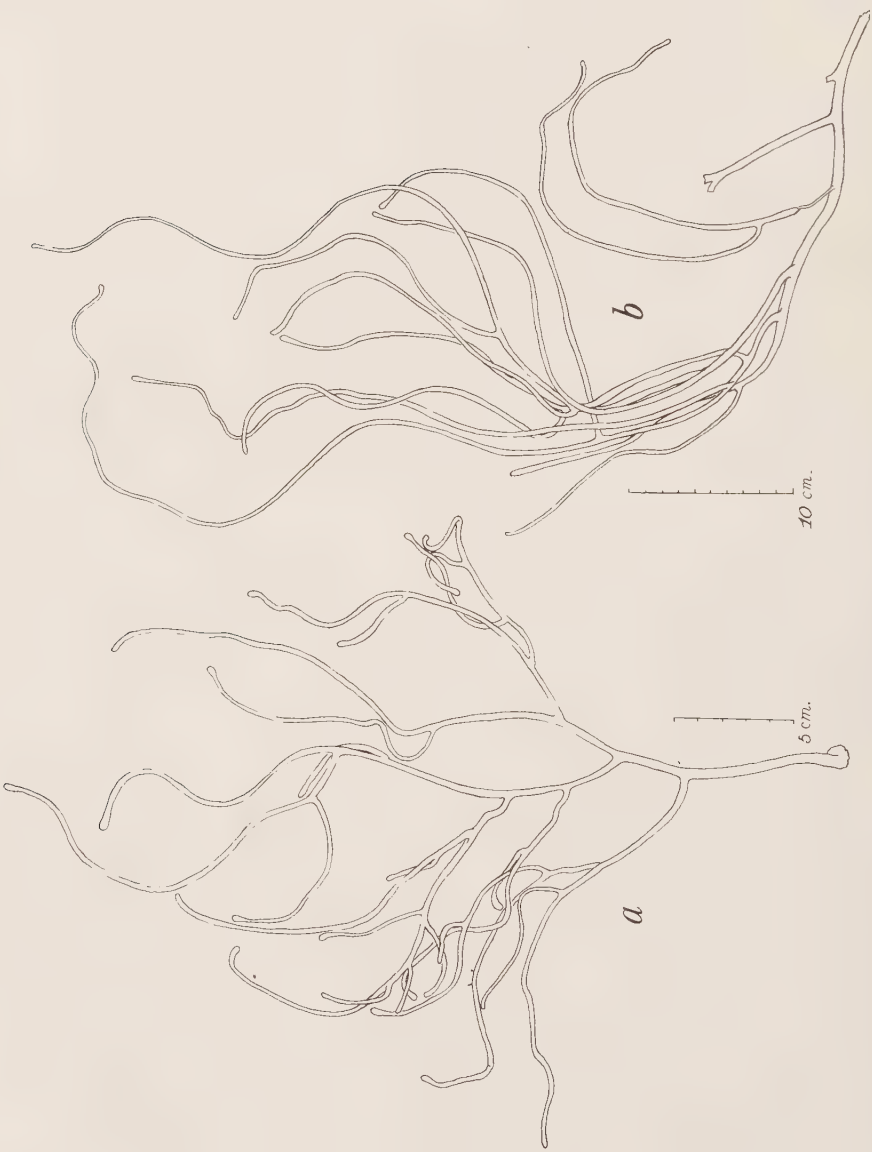


FIGURE 1. a, *Plexaura nina* sp. nov.; outline drawing of the type colony, reduced as indicated by 5 cm scale. b, *Eunicea pinta* sp. nov.; outline drawing of the type colony, reduced as indicated by 10 cm scale.

thal in 1924, only three, viz. *homomalla* Esper, its growth form *kükenthali* Moser, and *flexuosa* Lamouroux, remain in it as it is here defined; the remaining sixteen are redistributed among *Pseudoplexaura, Eunicea,* and *Muriceopsis.* A new species, closely related to *homomalla,* is here described.

Plexaura nina sp. nov.
(Figs. 1, a; 2; 3)

Diagnosis.—Colonies branched sparsely, dichotomously and in one plane, axillary angles 40°-90°. Terminal branches 1.5-2.0 mm in diameter and up to 25 cm long. Calicles verruciform, prominent everywhere except on the largest branches and the trunk. Anthocodiae with a crown of 4-6 bent rods *en chevron* beneath each tentacle and a collaret of curved rods in 2-3 transverse rows. Surface clubs small, foliate; tuberculate spindles of middle layer curved, up to 1.2 mm long; deep reddish purple capstans occur throughout the axial sheath, and bluntly spinose spindles in the terminal regions only. Color of rind grayish brown with white-bordered calicular orifices; anthocodiae white (in alcohol).

Description.—Colonies branched sparingly, in one plane; ramification mostly dichotomous, with axillary angles from 45° to 90°. The type is a colony 40 cm high including the base, with flexible, slender end branches up to 2 mm in diameter and 15 cm long. The branch tips are slightly clavate. Calicles are conspicuous over most of the colony, becoming low or quite flush with the surface of the rind on the trunk and major branches. Many of the anthocodiae are exsert in preservation (Fig. 2, a), being armed with a strong crown of 4-6 rods *en chevron* beneath each tentacle and a collaret of 2-3 rows of transverse rods; tentacles filled with smaller rods (Fig. 3, a). The outer layer of rind contains small leaf-clubs and asymmetrically foliate capstans 0.1-0.13 mm long (Fig. 2, h-m), which become stouter and coarser toward the base of the colony (Fig. 2, n-p). The middle layer of rind contains tuberculate spindles about 0.7 mm long near the tips of the branches (Fig. 3, d), 0.8-1.0 mm long toward the middle of the colony (Fig. 3, c), and up to 1.2 mm long in the main trunk (Fig. 3, b); most are white but a few of those nearest the axial sheath may be lavender. Some of the smaller spindles, especially those near the calicular margins, have one end enlarged and asymmetrically spinose (Fig. 3, e). The axial sheath contains dark purple capstans basically

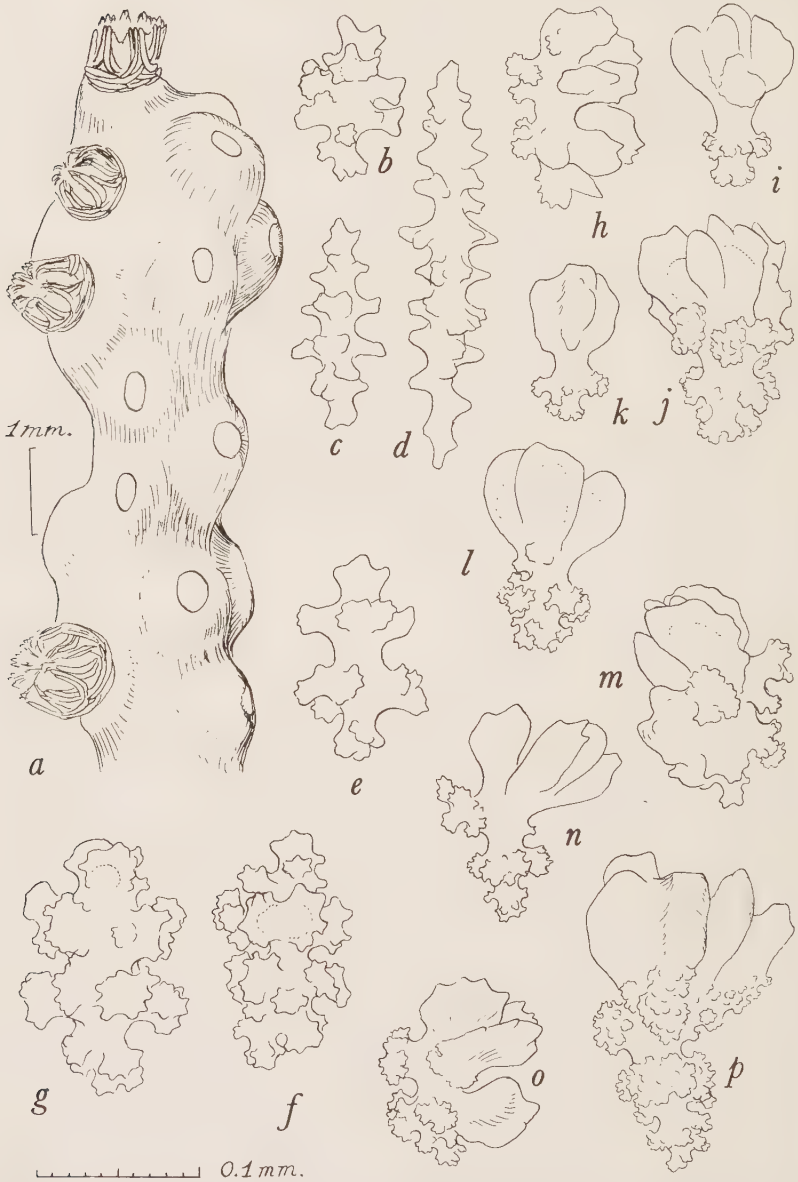


FIGURE 2. *Plexaura nina* sp. nov. a, branch tip drawn at low magnification, as indicated by 1 mm scale. b-d, deep purple spicules from the axial sheath layer of a terminal branch. e-g, deep purple spicules from the axial sheath layer of the main trunk. h-m, colorless clubs and foliate bodies from the surface layer of rind of a terminal branch. n-p, colorless clubs and foliate body from the surface layer of rind of the main trunk. (All spicules, b-p, drawn at high magnification as shown by 0.1 mm scale.)

FIGURE 3. *Plexaura nina* sp. nov. a, spicules of the crown: large, curved spindles of the collaret (right); terminally thorny spindles of the points (middle); small, flattened rods of the tentacle backs (left); all colorless. b, spindles from the middle layer of rind of the main trunk; white, white with purple core, or all purple; two clubs from surface layer drawn to same scale. c, spindles from the middle layer of rind near middle of colony; mostly white, a few pale purple. d, spindles from the middle layer of rind of a terminal branch: colorless; three clubs from surface layer drawn to same scale. e, asymmetrically spinose spindles from calicular margins. (All figures drawn at intermediate magnification as shown by 0.5 mm scale.)

of 8-radiate type, with blunt, complicated rays. They are rather small (length about 0.1 mm) in the terminal branches, where bluntly spinose spindles also occur (Fig. 2, b-e), but they increase in size and complexity basad, where there are no spindles (Fig. 2, f-g).

Color.—In alcohol, rind grayish brown, the calicular apertures with white borders; anthocodiae white.

Type.—U. S. National Museum, Cat. No. 50562: South tip of the Tongue of the Ocean, Great Bahama Bank, 23° 24′ 00″ North, 76° 33′ 00″ West, 36 fathoms, ALBATROSS Station 2649, April 12, 1886. Fragments in the Museum of Comparative Zoölogy, Cat. No. 2537.

Range.—Known at present only from the type locality.

Remarks.—Many features of *Plexaura nina* recall *Plexaura homomalla,* notably the strong anthocodial crown, the clubs of the surface layer, the spindles of the middle layer, and the capstans of the axial sheath. The outer clubs are smaller and less ornate than those of *homomalla,* but the coarser examples from low in the colony approach the *homomalla*-type in all features except size. The spindles of the middle layer of rind are like those of *homomalla* but are longer and more slender than usual in that species. The purple spicules of the axial sheath are similar in *P. nina* and *homomalla,* but spindles seem not to occur in the terminal branches of the latter. As usual, the spicules of all layers become larger and coarser toward the base of the colony, indicating that the secretory activity of the scleroblasts continues long after the spicules are fully formed. The colonial form of *P. nina* is more delicate than that of *homomalla,* the branching is less profuse and the branches are much more slender and flexible.

Genus *Eunicea* Lamouroux

Eunicea Lamouroux, 1816, p. 431. (Type species, *Eunicea mammosa* Lamouroux 1816, by subsequent designation: Bayer, 1955, p. 212.)
Eunicea Kunze, 1916, p. 505.
Eunicea Kükenthal, 1924, p. 118.
Eunicea Stiasny, 1935, p. 73.

Diagnosis.—Plexaurids with cortical spiculation divided into three layers: outer layer clubs (wart-clubs, thorn-clubs, leaf-clubs, or torches); middle layer predominantly spindles, often large and coarse (up to 3 mm long); inner layer (=axial sheath) with spindles or rods, some of which may develop into rudely sculptured ovoids toward base of colony. Invariably some spicules of the axial sheath are some shade of purple (deep red-purple, lavender, or violet), at least in young colonies and near the base of fully developed examples. Anthocodiae armed with spindles, rods, and flat rodlets, sometimes restricted to the tentacles, sometimes forming also a crown.

Remarks.—Kükenthal's account of this genus (1924), based largely on the work of Kunze (1916), is of little value today since it recognizes many species based upon differences in growth form and un-

reliable variations in spiculation. Stiasny's treatment (1935) is not much better from a systematic standpoint but contains more detailed and better illustrated descriptions.

The type species recognized by earlier workers, viz. *Eunicea succinea* (Pallas), is ineligible for designation as genolectotype because it was a doubtful species from the standpoint of the author of the genus (Int. Rules of Zool. Nomenclature, Art. 30 (e)β). A new selection was accordingly made by Bayer (1955) as noted in the synonymy above.

In our opinion, the genus *Eunicea* is not homogeneous, but divides naturally into two groups of species, the first including those related to *Eunicea mammosa* Lamouroux (*Eunicea* s.s.), the second those related to *E. tourneforti* Milne Edwards and Haime. The latter group was called *Euniceopsis* by Verrill (1907), a name that we propose to recognize in a subgeneric sense.

The species of the subgenus *Eunicea* s.s. are characterized by weak anthocodial spiculation concentrated in the tentacles (excluding the pinnules) and extending little if at all onto the body wall below the tentacles (except for the new species described below, which has a weak crown of one or two pairs of rods *en chevron* in each sector); the outer clubs have long handles and ornately laciniated heads, producing the type we call "torches;" and most or all of the spicules of the axial sheath are invariably deep reddish purple throughout the colonies.

The species of the subgenus *Euniceopsis* Verrill are characterized by strong anthocodial spiculation consisting of small, flat rods in the pinnules, larger rods in the tentacle backs, and large, bent spindles forming a crown in the body wall below the tentacles; the outer clubs have short handles and take the form of leaf-clubs or wart-clubs, rarely thorn-clubs; and the spicules of the axial sheath are colorless and either lavender or pale violet but never dark purple.

There is evidence suggesting that *Euniceopsis* may eventually require subdivision to form a third subgenus within *Eunicea* s.l. Several of its species have a well-developed collaret formed by the transverse or oblique orientation of the proximal crown spicules, whereas others have no collaret but only eight well-separated points with the proximal spicules nearly or quite longitudinal. In the latter group, of which *E. tourneforti* is typical, the pale violet spicules of the axial sheath seem to be formed only during the early development of the colonies, all spicules formed after a certain age being colorless, since they (1)

appear throughout small colonies, (2) are concentrated in the basal region of larger specimens, and (3) are proportionally fewer in larger colonies. In the former group, however, numerous lavender spicules occur in the axial sheath at all levels, regardless of the size of the colonies.

Eunicea (*Eunicea*) *pinta* sp. nov.
(Figs. 1, b; 4; 5)

Diagnosis.—Colonies with long, slender branches (diameter 2.0-2.5 mm, length 50 cm) arising both laterally and dichotomously. Anthocodiae with a crown of at least one pair of bent rods beneath each tentacle, and rodlets transverse in tentacle backs but not extending into pinnules. Calicles not projecting, margins 8-lobed. Surface clubs as torches 0.13-0.16 mm long, with large, spiny heads and slender handles; middle layer of rind with spinose needles and slender tuberculate spindles 1.0-1.8 mm long; axial sheath with bright purple spindles and capstans sculptured with conical processes.

Description.—The growth form is tall and straggling, with slender, flexible branches arising laterally and dichotomously, usually at quite a wide angle, from 45° to 90°, with a tendency to remain in one plane (Fig. 1, b). The tallest colony measures 80 cm in height and has unbranched terminals as long as 50 cm. The main trunk is 7-10 mm in diameter, and the terminal branches, which are more or less clavate, measure 2.0-2.5 mm in diameter. They are cylindrical, smooth, and have only the slightest hint of elevation around the calicular orifices, which have eight marginal lobes but never a projecting calicle or lower lip. The anthocodiae, many of which are preserved exsert, have a crown of slender, curved rods about 0.45 mm long, at least one pair and sometimes two or three pairs below each tentacle, and numerous small flat rodlets 0.15-0.2 mm long placed transversely in the tentacle backs (Fig. 5, d). The outer layer of rind contains a dense layer of very spinose torches with large heads and slender handles, about 0.15 mm in length (Fig. 4, d-e), unlike those of any other known species. Presumably the oldest torches, near the base, are the largest, their size decreasing toward the branch tips. The middle layer of rind contains a great abundance of extremely slender, spinose needles 0.4-0.5 mm long, and some slim, curved spindles with compound tubercles (Fig. 5, a). In the main trunk, the largest spindles measure about 1.8 x 0.2 mm (Fig. 5, c); they decrease in length and stoutness toward the

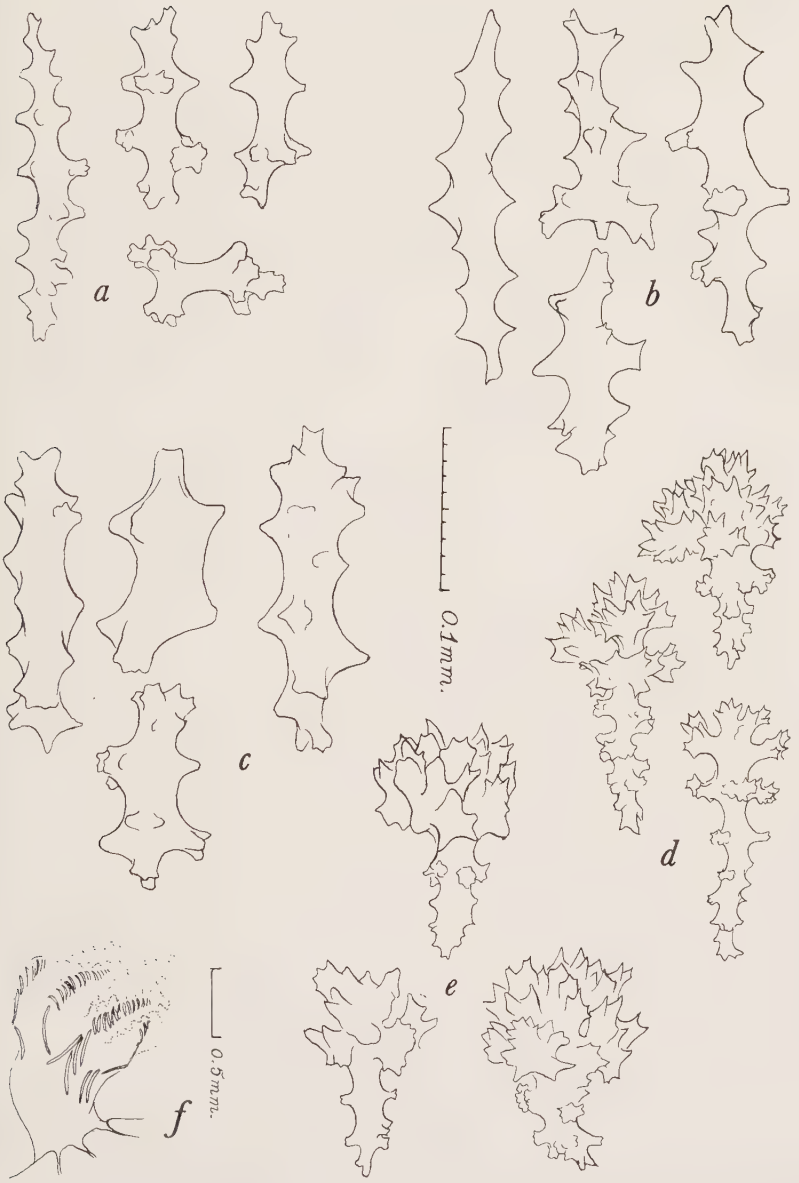


FIGURE 4. *Eunicea* (*Eunicea*) *pinta* sp. nov. a, purple spicules from the axial sheath of a terminal branch. b, purple spicules from the axial sheath near middle of colony. c, purple spicules from the axial sheath of the main trunk. d, colorless torches from the outer layer of rind of a terminal branch. e, colorless torches from the outer layer of rind of the main trunk. f, expanded polyp showing arrangement of spicules in the crown. (All spicules, a-e, drawn at high magnification as shown by 0.1 mm scale; polyp drawn at low magnification as shown by 0.5 mm scale.)

FIGURE 5. *Eunicea pinta* sp. nov. a, colorless needles and spindles from the middle layer of rind of a terminal branch. b, white spindles from the middle layer of rind from middle of colony; two torches of surface layer drawn to the same scale. c, white spindles from the middle layer of rind of the main trunk (sculptural detail omitted from largest); two torches drawn to the same scale. d, spicules of the crown. (All spicules drawn at intermediate magnification as shown by 0.5 mm scale.)

upper part of the colonies, in the middle region reaching 1.2 x 0.15 mm (Fig. 5, b), and at the branch tips about 1.0 x 0.05 mm. The slender needles are abundant throughout. The torches, spindles and needles are entirely colorless. The innermost layer of rind (the axial sheath) contains exclusively purple spicules: at the branch tips they are spinose spindles about 0.2 mm long, and slender capstans with two whorls of spines, mostly about 0.13 mm in length (Fig. 4, a); toward the middle of the colony, the spindles attain a length of about 0.22 mm and the capstans about 0.15 mm (Fig. 4, b); near the base, there are blunt rods about 0.18 mm in length, and stubby capstans about 0.12 mm (Fig. 4, c), but no acute spindles.

Color.—In alcohol, white; axis dark brown.

Type.—U. S. National Museum, Cat. No. 50563: South tip of the Tongue of the Ocean, Great Bahama Bank, 23° 24′ 00″ North, 76° 33′ 00″ West, 36 fathoms, ALBATROSS station 2649, April 12, 1886. Fragments in the Museum of Comparative Zoölogy, Cat. No. 2536.

Range.—Known at present only from the type locality.

Remarks.—The spicular characters of *Eunicea pinta* definitely point to an affinity with the *mammosa* group of species. The straggling, flexible growth form indicates an adaptation to conditions less turbulent than those that obtain in shallow water on the reefs.

REFERENCES

BAYER, FREDERICK M.
1955. Contributions to the nomenclature, systematics, and morphology of the Octocorallia. Proc. U. S. nat. Mus., *105:* 207-220, 8 pls.

KÜKENTHAL, WILLY
1924. Gorgonaria. Das Tierreich, *47.* xxviii+478, 209 figs. Berlin.

KUNZE, G.
1916. Die Gorgonarien Westindiens: Kap. 4, Die Gattung Eunicea Lamouroux. Zool. Jb., Suppl., *11:* 505-552, 25 figs., pls. 24-26.

LAMOUROUX, J. V. F.
1812. Sur la classification des polypiers coralligènes non entièrement pierreux. Nouv. Bull. Sci. Soc. Philom., *3:* 181-188.
1816. Histoire des polypiers coralligènes flexibles, vulgairement nommés zoophytes. lxxxiv+560, 19 pls. Caen.

MOSER, JOHANNES
1921. Ergebnisse einer Revision der Gattung Plexaura Lamouroux. Zool. Anz., *53:* 110-118.

STIASNY, GUSTAV
1935. Die Gorgonacea der Siboga-Expedition. Supplement I, Revision der Plexauridae. Siboga Exped., Monogr. *13b*[7]. vi+106, 27 figs. 7 pls.

VERRILL, ADDISON EMERY
1912. The gorgonians of the Brazilian coast. J. Acad. nat. Sci. Philad., *15* (2): 373-404, 1 fig., pls. 29-35.

A REVIEW OF CIGUATERA, TROPICAL FISH POISONING, WITH A TENTATIVE EXPLANATION OF ITS CAUSE[1]

JOHN E. RANDALL
The Marine Laboratory, University of Miami

Abstract

The categories of fish poisoning as proposed by Halstead and Lively (1954) are revised. An attempt is made to document what appear to be the established phenomena of ciguatera, an illness of occasional occurrence following the ingestion of various tropical reef and inshore fishes and possibly certain echinoids and gastropods. The toxin appears to be cumulative and the most toxic fishes, generally, are large piscivorous types like barracuda, jacks, and groupers. Plankton-feeding fishes have not been implicated in ciguatera. Herbivorous and detritus-feeding fishes and mollusk-feeders may be poisonous. Fishes causing ciguatera are not found universally over large areas, but are localized, often in small sectors. A region once poisonous may lose its poisonous fishes and vice versa. Previous theories of the cause of ciguatera are discussed, and a new hypothesis is presented. In this, it is assumed that a benthic organism, most likely a blue-green alga, is the source of the toxin. This organism would seem to be one of the first growing in normal ecological succession in tropic seas. The localization of poisonous fishes is explained in terms of availability of new substratum for marine growth. Recommendations are made for further reasearch on ciguatera.

Introduction

Certain marine fishes have long been known to cause illness, and on occasions death, when eaten by man. Some cases of fish poisoning have undoubtedly been due to toxins produced by bacterial action on the fish following their capture. However, numerous accounts by scientific observers clearly indicate that toxins may be present in the flesh and viscera of freshly-caught fish.

Halstead and Lively (1954: 165-167) have separated poisonings from eating marine fishes into four groups on the basis of symptomatology: ciguatera; tetraodon poisoning; gymnothorax (moray eel) poisoning; and scombroid (tuna) poisoning. Ciguatera is a usually nonfatal illness of occasional occurrence following the ingestion of various tropical reef and semi-pelagic fishes such as barracuda (Sphyraenidae), snappers (Lutjanidae), groupers (Serranidae), surgeonfishes (Acanthuridae), jacks (Carangidae), and possibly certain edible gastropod and echinoid invertebrates. It appears to be the same throughout the tropics, although its origin from a single toxin awaits

[1]Contribution No. 212 from The Marine Laboratory, University of Miami.

demonstration. Tetraodon poisoning is a severe, sometimes fatal toxemia caused by puffers and porcupine fishes, the ovaries and other internal organs of which are especially toxic. It is distinct and well-documented (for a review, see Yudkin, 1944). Further evidence is needed, however, to recognize gymnothorax and scombroid poisonings as different from ciguatera or not due to bacterial contamination. It is the belief of the author that gymnothorax poisoning is merely severe ciguatera, and examples of scombroid poisoning seem to be primarily bacterial in origin. Before discussing these dubious categories of fish poisoning further, it is advisable to present the characteristics of the ciguatera syndrome.

The symptoms of ciguatera appear from about one to ten hours after toxic fish are eaten; those most commonly given for the illness are: weakness or prostration, diarrhea, tingling or numbness of lips and hands and feet, confusion of sensations of heat and cold, nausea, joint and muscular pain, inability to coordinate voluntary muscular movements, difficulty in breathing, burning urination, and itching. Probably the most diagnostic symptoms are the tingling sensations in the hands and feet, frequently described as like "pins and needles", or "electric shocks", and the feeling of heat when cold objects are touched or cool liquids are taken into the mouth. Light cases may not exhibit these sensations, however. The author was mildly poisoned in the Tuamotus by eating a small amount of a 500 mm grouper (*Plectropomus leopardus*) which was cooked immediately after capture. Only weakness, especially of the lower limbs, and diarrhea were evident. If two associates who ate large amounts of the same fish had not acquired typical ciguatera, the slight sickness of the author would not have been attributed to the ingestion of the fish. The gastrointestinal distress of ciguatera is usually of short duration, but the neural symptoms may last for many weeks. Various persons in the Society Islands who had suffered from ciguatera informed the author that the tingling sensations of the extremities may be intensified and prolonged by eating reef fishes which are nontoxic to individuals not in the recovery phase of the illness. In reference to ciguatera in the Caribbean, Brown (1945: 34) wrote, "The effects may last for three months, and as long as the symptoms are present, any fish eaten will make them worse."

Halstead and Lively stated that gymnothorax poisoning develops more rapidly than ciguatera, is more violent, and convulsions and paralyses are prominent. The references from which these authors

concluded that gymnothorax poisoning is unique appear to be only Khlentzos (1950) and Ralls and Halstead (1955). Khlentzos reported on poisoning of 57 men on Saipan from eating a single moray eel (*Gymnothorax flavimarginata*) six feet in length and about one foot in thickness. Twenty minutes after the meal, they experienced tingling and numbness about the lips and tongue. In spite of gastric lavage, severe neurotoxic symptoms developed; 14 patients became comatose, and two died. Ralls and Halstead summarized similar case histories of six men poisoned (one fatally) by the same species of eel in the Marshall Islands. Belotte (1955) presented a case history of a 40 year old American who was hospitalized with ciguatera in Tahiti after eating a large specimen of the snapper *Lutjanus monostigmus*. This man was in a coma three days, and his illness seemed comparable to those persons poisoned severely by moray eels. Two men in the Society Islands who became sick, but not as violently as the above individuals, following the ingestion of a moray eel (*Gymnothorax* sp.) related their symptoms to the author. Both men were very weak, had diarrhea, tingling sensations, and pain, especially in the joints. These symptoms are typical of ciguatera.

Halstead (1954) has pointed out that scombroid poisoning is a histamine-type intoxication and not a neurotoxic one like ciguatera. In most instances the cases followed the eating of tuna, bonito, or skipjack which were a day or more old. Phisalix (1922: 607-608) wrote that the species belonging to the genera *Scomber, Thunnus,* and *Cybium* are remarkable for the rapidity with which their flesh alters after they are caught; unless they are "thrown from the net into boiling water" they can cause illness from bacterial action which could pass as fish poisoning. Halstead (1954) reported an atypical outbreak of scombroid poisoning at Johnston Island from *Euthynnus yaito*. The symptoms were identical with those of ciguatera. This small tuna is not exclusively pelagic; it is frequently encountered over reefs and may enter lagoons and bays.

Undoubtedly more valid categories of illness from eating marine fishes exist besides ciguatera and tetraodon poisoning. Probable examples are toxic cyclostomes, chimaeras, elasmobranchs (especially the liver of sharks), *Tetragonurus cuvieri,* certain tropical clupeid fishes, *Ruvettus pretiosus,* the roe of *Scorpaenichthys marmoratus,* trunkfishes (Ostraciidae), and the head of the goatfish *Upeneus arge*. Some of these other categories will be discussed more fully before dealing with ciguatera in detail.

Information is not yet available to demonstrate whether or not tropical reef sharks cause ciguatera. The toxicity of the liver of sharks, at least of pelagic species, may be due to the high vitamin A content (Wheeler in Wheeler and Ommanney, 1953: 47). Jensen (1914, 1948), Bϕje (1939), and Hjortland (1917) have reported some of the arctic sharks to be poisonous (references from Halstead and Lively, 1954).

On at least two occasions Risso (1810) endured intense gastric pain, abdominal swelling and a sensation of heat in the throat after eating *Tetragonurus cuvieri* in the Mediterranean. He attributed the toxicity of this fish (believed to be a deep-water species, but has been taken at the surface) to its feeding on the siphonophore *Stephanomia*. Fitch (1952) was unable to demonstrate the toxicity of four specimens of *Tetragonurus* from California; stomachs of these fish contained unidentified coelenterates and ctenophores. Phisalix (1922: 587) (after Moreau de Jonnès) cites *Mola mola* as sometimes toxic, as does Mills (1956: 102). It also is known to feed on jellyfishes.

The eating of certain tropical clupeid fishes has caused an illness, sometimes said to be seasonal, which is often violent and commonly fatal. *Pacific Islands Monthly* (Jan. 1956: 142) reported five deaths in Fiji from eating a local herring. Stephenson (1838: 122) gave the symptoms following the ingestion of the thread herring *Megalops thrissa* (=*Opisthonema oglinum*) of the Caribbean as nausea, vertigo, extreme heat and itching, violent pain in the stomach, tenesmus, great acceleration of pulse, cold sweats, insensibility, and convulsions. Death may occur within a half hour; survivors may be jaundiced. Strong (1944: 1545) stated that *Meletta venenosa* (considered a synonym of *Harengula punctata* by Fowler, 1928: 31) in New Caledonia has caused painful cramps, dyspnoea, cyanosis, cold sweats, dilated pupils and at times death (for a report of the autopsy of a man who died after eating this herring and records of other poisonous clupeoid fishes, consult Phisalix, 1922: 599-601, 612). While discussing clupeid poisoning with the author, Donald de Sylva made the suggestion that there may be a connection between blooms of the tropical planktonic blue-green alga *Skujaella* (*Trichodesmium* in the older literature) and the toxicity of herrings (which are plankton-feeders). If abundant, this alga produces discolored water at sea, and when wind-driven ashore has caused mortality of shallow-water fishes and invertebrates (Veenhuyzen, 1879; Mohler, 1941).

Hubbs and Wick (1951) have demonstrated the toxicity of the

roe of the cabezone (*Scorpaenichthys marmoratus*) in California, and Pillsbury (1957) has observed that egg masses of this cottid fish are avoided by predators in British Columbia.

In reference to toxic trunkfish (believed to be *Lactophrys bicaudalis*), Brown (1945: 36) stated, "the poison is localized in small pockets of jelly on each side within the carapace just behind the gill opening. Market vendors in Nassau, Bahamas, always cut this out before selling this fish: the symptoms are unsteadiness in gait similar to drunkenness (Clarke, 1920) and the effects may be serious."

The brain of the goatfish *Upeneus arge* in Hawaii is said to have a poisonous quality. Those who eat it have restless sleep, nightmare, or a sort of delirium. There is a sensation of loss of balance and especially a feeling that the head is lower than the feet (Titcomb, 1952: 136-137).

According to Poey (1866) the word ciguatera was first applied to an illness resulting from eating the marine snail *Livona pica* (a staple seafood throughout the Caribbean) called cigua in Cuba, and later to gastrointestinal-neural upsets following the ingestion of various fishes. It has since been used by some authors for non-tetraodon ichthyotoxism in the tropical Indo-Pacific area where the effects of the poisoning seem to be the same as in the Atlantic.

The importance of ciguatera goes beyond the purely medical aspect of treatment of patients suffering from the ingestion of toxic fish. Many nontoxic fish are denied to humans or domestic animals as food because of fear of their being poisonous. Sections of reef with a reputation of harboring poisonous fishes are not fished, whereas nontoxic areas may, as a result, be overfished.

Although ciguatera has been recognized for centuries, its origin is still unknown, treatment is only symptomatic, and a test for toxicity of fish is yet to be devised. Arcisz (1950: 19) wrote, "An extensive survey of the literature on fish poisoning discloses that very little is definitely known regarding this phenomenon except the symptomotology, a few of the species of fish involved, and the localities where the poisoning is prevalent."

Numerous theories have been proposed to explain how fishes become poisonous. One of the most unique ascribes poisonous qualities to fish left in moonlight. Some authors claim that the toxin is endogenous. This is probably true for the puffers but not for fishes causing ciguatera, because it fails to explain why a species of fish is poisonous in one area and not another.

Many authors have assumed that fishes become toxic by virtue of their food habits. The following have been suspected as a basis of ciguatera due to ingestion by fishes: berries from the poisonous machineel tree, algae, dinoflagellates, corals, jellyfishes, palolo worms, mollusks, and other invertebrates. Co-authors of two recent papers have favored benthic algae as a source of the toxin. Dawson, Aleem, and Halstead (1955) have commented on the presence of algae in the stomach contents of many poisonous fishes, and Habekost, Fraser, and Halstead (1955) found that most of the algae they tested from Palmyra and California were toxic. Conclusions, however, were based on an unreliable method of injecting extracts into mice (see below). Furthermore, since California has no poisonous fish problem, whatever is allegedly toxic in algae at this locality at least is not capable of producing ciguatera. Hutner and McLaughlin (1958: 96, 98) have suggested that the toxin causing fish poisoning may be produced by zooxanthellae in corals. When cultured free of coelenterates, one of these algae became a mobile dinoflagellate. Although this species is not toxic, these authors pointed out that other symbiotic dinoflagellates may be, and fishes feeding on coral containing them may become poisonous in turn. This hypothesis does not explain how fishes which do not feed on corals or on animals that feed on corals may still cause ciguatera when eaten.

There is a persistent belief that some chemical substance contaminates the environment, thus resulting in ciguatera. Copper is most frequently implicated, primarily because copper-bottomed ships and naturally-occurring copper deposits have long been associated with poisonous fishes. Proponents of such a theory would find it difficult to explain why poisonous fishes are found away from a source of the chemical, or why only some and not all of the fishes in a contaminated area are toxic. Ross (1947) stated that poisonous fishes were first noted at Fanning Island in the Central Pacific four months after war materials were dumped by the American army in 1945. The fishes were toxic in the vicinity of the dumping site. He thought that plankton became poisonous from the deterioration of the metals (especially copper), and the fishes, ultimately, by eating toxic plankton. Like some other authors (Lee and Pang, 1944; Mills, 1956), he endeavored to draw an analogy between ciguatera and mussel and clam poisoning which results from contamination of the pelecypods with toxin from the planktonic dinoflagellate *Gonyaulax*.

De Sylva (1956: 5) suggested that the great barracuda (which

seems to cause ciguatera more than any other fish in the Caribbean region) might become poisonous by eating puffers, boxfishes, and the like. To explain the different symptoms of puffer and barracuda poisoning he wrote, ". . . it is not inconceivable that the poison absorbed by the barracuda in consuming its prey undergoes biochemical change." This theory does not seem correct because fishes which do not prey on puffers or any other species of fish may produce ciguatera when eaten.

In this paper a tentative hypothesis is offered in an effort to explain the origin of ciguatera toxin and to integrate what appear to be the established phenomena associated with the illness. This is followed with a suggested pattern for future research. Before presenting the hypothesis, the existing knowledge of ciguatera is summarized in the discussion section below, along with field observations, primarily from the Pacific.

While the author was in the Society Islands studying the biology of certain serranid and lutjanid fishes for Yale University, the Bernice P. Bishop Museum, and the Division of Fish and Game, Territory of Hawaii, a request was received from A. H. Banner, Director of the Hawaii Marine Laboratory, for information on poisonous fishes. He wished an assessment of the value of the Tahiti area as a site for a future major study of ciguatera. In fulfilling the request, emphasis was placed on case histories of persons poisoned by eating fishes, interviews with local French and Tahitian people, whose aid is gratefully acknowledged, and on procurement of toxic fishes for examination at the Hawaii Marine Laboratory. In arriving at the conclusions contained herein, the author is most indebted to Dr. Banner, who helped in many ways. Others to whom thanks are due for assistance and information are P. D. F. Palmer of Fanning Island, Michio Takata of the Division of Fish and Game, Territory of Hawaii, Vernon E. Brock, Garth I. Murphy, and D. W. Strasburg of the Pacific Oceanic Fishery Investigations of the United States Fish and Wildlife Service, W. Jan Newhouse of Hawaii, Elgin Forsythe of Nassau, Bahamas, Donald S. Erdman of Puerto Rico, Conrad Limbaugh of the Scripps Institution of Oceanography, Donald de Sylva of the University of Delaware, and Bruce W. Halstead of the United States Navy.

Discussion

The toxin causing ciguatera has not been isolated. It is resistant to drying and is thermostable, for illness follows the ingestion of toxic

fish whether raw or cooked. After an outbreak of poisoning from a barracuda in Japan, Hashimoto (1956) extracted the flesh of the fish in methyl alcohol at pH 3.8. The alcohol was removed and the residue extracted with ether. The fat-free residue had no effect when fed to a cat, but the oily substance in the ether-soluble fraction, after removal of the ether, proved fatal to another cat. The toxin was found to be soluble in acetone and hot water but not in tartaric acid at pH 2.8. Banner and Boroughs (MS, see references), working with toxic red snapper (*Lutjanus bohar*) from Palmyra, Line Islands, have demonstrated that the toxic substance may be extracted from dehydrated ground flesh in 90% ethanol (less efficiently in 70% ethanol and very little in absolute alcohol). It is detoxified if concentrated in normal atmospheric oxygen. When the alcohol solution is evaporated to dryness under nitrogen and extracted with chloroform or diethyl ether, toxicity is observed only in the chloroform and diethyl ether solutions.

At present the only means of detecting toxins in fish involves the use of experimental animals. Banner and Boroughs have shown that the method of homogenizing portions of fish in water, centrifuging, and injecting the supernatant intraperitoneally into mice (Halstead and Bunker, 1954a) is not reliable, for the toxin is not soluble in water, and toxicity depends upon matter suspended in the extract. Banner and Boroughs (MS) and Takata (MS) will discuss further the lack of dependability of this method. Actual feeding of fish to the test animals is preferred. Cats are sensitive to ciguatera toxin and have often been used in feeding experiments. Banner and Boroughs have found mongooses in Hawaii to be more suitable, for they do not regurgitate toxic fish, whereas cats often do. Mice showed no symptoms when fed toxic fish even at twice the dose in percentage of body weight that was lethal to a mongoose.

Many authors have corroborated the widespread belief that larger fish are more apt to be poisonous than smaller individuals of the same species. Banner and Boroughs (MS) and Takata (MS) will offer supporting evidence from feeding experiments with animals.

The toxin is not rapidly dissipated in fishes when removed from the source material. Takata maintained poisonous groupers and snappers from the Line Islands on nontoxic food in aquaria on Oahu, Hawaiian Islands, where there is little or no ciguatera problem from local fishes. He found some capable of eliciting extremely severe symptoms when fed to cats after periods of aquarium life up to 14 months.

There have been claims that fish poisoning is seasonal, and this has been attributed by some to the development of the gonads (Fish and Cobb, 1954: 6). Arcisz (1950: 9, 20, Table 4) has tabulated the occurrence of 26 recorded outbreaks of fish poisoning from various species in Puerto Rico and the Virgin Islands by month and concluded that the poisoning is not seasonal. Ross (1947) reported cases of ciguatera from Fanning Island, Line Islands from February, 1946 to April, 1947 in every month but August. Belotte (1955) treated ciguatera patients in Tahiti for three years. He stated that the illness may occur any time of the year, and the quality of the flesh of fish is not modified during the spawning season.

The toxicity varies in the different tissues of a poisonous fish; generally the liver and alimentary tract are more toxic than somatic muscle. The author is not in agreement with Halstead and Bunker (1954a: 9) who stated that the higher concentration of toxin in the liver and intestine may be due to these organs receiving the toxin first following a meal containing poisonous substance. They suggested that fish removed from the source of toxin would show a reversal of relative toxicity of viscera and somatic muscle due to detoxification of the active principle in the liver and intestine. First of all, no one has demonstrated that ciguatera toxin is toxic to the fish. It is certainly evident that it affects fishes far less than the mammals which have been tested. Takata's experiment demonstrates that detoxification, if it occurs at all, proceeds very slowly. There is no evidence to support the speculation of reversal of toxicity in the tissues of fish, but there is some to the contrary. While the author was in the Society Islands a Tahitian man who fed upon the liver of a poisonous grouper (*Cephalopholis argus*), maintained for 17 days in a wire enclosure in the sea on nontoxic food, was much more ill than four other individuals who ate only the flesh of this and other fish of this species from the same area.

Numerous fishes have the capacity to produce ciguatera when eaten. Often the same genera and not infrequently the same species of fishes are involved in tropical fish poisoning throughout the vast Indo-Pacific region, as will be seen from the following accounts.

Belotte (1955) listed the snapper *Lutjanus monostigmus*, the grouper *Plectropomus leopardus*, the surgeonfish *Ctenochaetus striatus*, and triggerfishes (Balistidae) as commonly poisonous in Tahiti. *L. monostigmus* was regarded as the most dangerous species; its sale is forbidden in the Papeete market. The groupers *Epinephelus elon-*

gatus and *Cephalopholis argus,* the mullet *Crenimugil crenilabus,* and various parrotfishes (*Scarus* spp.) were also implicated. From interviews with Tahitian people, the author is in agreement with Belotte on the preceding, but would add as probably equivalent in potential toxicity to *L. monostigmus,* two other large snappers, *L. bohar* and *L. rivulatus* (both uncommon in Tahiti), and two or three large species of moray eel (*Gymnothorax*). *Sphyraena barracuda,* the lutjanoids *Lethrinus miniatus* and *Monotaxis grandoculis,* large jacks (*Caranx* spp.), the grouper *Variola louti,* and the giant wrasse *Cheilinus undulatus* are also capable of being moderately to strongly toxic. In especially toxic sectors, many other kinds of fishes, such as species of surgeonfishes of the genus *Acanthurus,* may be poisonous.

From interviews with natives, Harry (1953: 177) recorded the following fishes as dangerous to eat at Raroia, Tuamotu Archipelago: *Lutjanus bohar, Lutjanus vaigiensis, Plectropomus leopardus, Sphyraena barracuda, Ctenochaetus striatus,* and balistids (all scientific names altered to comply with Schultz *et al.,* 1953).

Ross (1947), the medical officer on Fanning Island when there were 95 cases of ciguatera among a population of 224 people in slightly more than one year, listed the fishes which were responsible by local English, Gilbertese, and some haphazard scientific names. The Gilbertese names (Randall, 1955a) were the most useful in tying down the following probable species names: *Caranx lugubris, Cephalopholis argus, Epinephelus* spp., *Aprion virescens, Lutjanus gibbus, Crenimugil crenilabis, Albula vulpes,* and *Cheilinus undulatus.* In addition there were surgeonfishes and an unidentified fish called bream (possibly *Monotaxis grandoculis*).

Hiyama (1943) reported on poisonous species in the Marshall and Mariana Islands. He tested fishes by feeding them to cats, mice, and puppies, and he augmented his experimental results with opinions of local fishermen. He cited the following as strongly toxic: *Gymnothorax* spp., *Sphyraena barracuda, Caranx melampygus* (when large), *Caranx lugubris* (when large), *Lutjanus gibbus, Lethrinus* spp., *Monotaxis grandoculis* (when large), *Gnathodentex aurolineatus* (one specimen tested), *Coris gaimardi, Plectropomus oligacanthus, Variola louti, Epinephelus fuscoguttatus,* and *Odonus niger. Balistoides niger* and the intestine of the file fish *Alutera scripta* were said by natives to be very poisonous. Listed as mildly toxic were: *Sphyraena forsteri, Lutjanus bohar, Aprion virescens, Lethrinus* spp., *Epibulis insidiator, Cephalopholis argus, Plectropomus truncatus, Epinephelus* spp.,

Ctenochaetus striatus, Ctenochaetus cyanoguttatus, and *Acanthurus gahhm* (questioned). Among those regarded as slightly toxic were *Lutjanus monostigmus, Lutjanus vaigiensis, Scarus microrhinos,* and *Cheilinus* spp. Hiyama's color plates facilitated the confirmation of scientific names.

Jordan (1929) has stated that the principal fishes which may produce ciguatera in Samoa are *Lutjanus bohar, L. monostigmus, Lethrinus miniatus, Cephalopholis argus, Acanthurus lineatus,* large morays (*Gymnothorax*), triggerfishes (Balistidae), filefishes (Monacanthidae), and trunkfishes (Ostraciidae). He also documented the fishes that are believed always good to eat in Samoa. Some of the latter have been implicated by other authors as poisonous elsewhere, although usually they are not indicated as strongly toxic. Many of the fishes which Jordan listed as safe to eat, such as mojarras (Gerridae), squirrelfishes (Holocentridae), scad (*Trachurops*) (=*Selar*), halfbeaks (Hemirhamphidae), flyingfishes (Exocoetidae), silversides (Atherinidae), and goatfishes such as *Mulloidichthys samoensis,* seem to enjoy a reputation for being nonpoisonous wherever they occur.

From Whitley (1943) it may be inferred that *Lutjanus coatesi* (probably =*L. bohar*), and *Paradicichthys venenatus* have been involved in ciguatera from the Great Barrier Reef in Australia.

Although Wheeler (in Wheeler and Ommanney, 1953: 31-33, 44-48) believes fish poisoning is due to "ptomaines" rather than "inherent qualities in the fishes", it would appear that the following species discussed by him have caused ciguatera in Mauritius and other islands in the Indian Ocean: *Lutianus civis* (=*Lutjanus bohar*), *Lutjanus rivulatus, Genyoroge melanura* (=*Lutjanus gibbus*), *Plectropomus maculatus, Variola louti, Epinephelus* spp., *Lethrinus harak, Siganus* (=*Teuthis*) *oramin, Teuthis rostrata,* and *Caranx.*

The West Indian area of the Atlantic is the site for numerous records of fish poisoning. Although most of the species of fishes which are involved are different from those in the Indo-Pacific, the same groups predominate—barracuda, jacks, groupers, and snappers.

Arcisz (1950) has tabulated the following fishes from the literature as having caused ciguatera in the West Indies: *Sphyraena barracuda;* the kingfish and cero (*Scomberomorus* spp.); six species of *Caranx;* the amberjack (*Seriola falcata*); the yellow-fin and tiger groupers (*Mycteroperca* spp.), *Epinephelus morio,* the hogfish (*Lachnolaimus maximus*), and the red snapper *Lutjanus blackfordi* (possibly confused

with another red snapper). Poey (1866) presented a list of poisonous fishes, primarily from Cuba. Among those not mentioned by Arcisz which should perhaps be included among the more dangerous Atlantic species are the black-fin grouper (*Mycteroperca bonaci*), the dog snapper (*Lutjanus jocu*), and the green moray eel (*Gymnothorax funebris*).

If an analysis is made of the kinds of fishes which have caused ciguatera and those that have not, especially in the light of our knowledge of their habitats and food habits (admittedly limited for many species), certain tentative conclusions emerge.

The fishes are usually bottom-dwelling but may be open-water forms. In either case they are shore fishes; usually they are found at depths less than 200 feet. A possible exception arises from the poisoning of 41 men (reported by Cohen, Emert, and Goss, 1946) in the Marianas by a 55 pound barracuda-like fish said to be the oil fish or escolar (*Ruvettus pretiosus*) which lives at depths of about 300 to 400 fathoms. The symptoms of the sick men were clearly those of ciguatera. Identification of the fish was provided from a "rough sketch" and "inadequate description" by L. P. Schultz who wrote of the occasion (in Schultz and Stern, 1948: 70-71), "The guesses of the people on the spot were that the offender was a barracuda. The statement was made by a Japanese doctor that the fish had four dog teeth hanging down at the front of its upper jaw." On the basis of the teeth Schultz suspected the oil fish. Actually this remark on dentition suggests a barracuda (contrast the photograph of a barracuda skull in Figure 7 of Whitley, 1943, which shows four huge canines anteriorly in the upper jaw, with the dentition of *Ruvettus pretiosus* shown in Figure 210 of Goode and Bean, 1895; the anterior canines of the latter are not markedly longer than more posterior teeth). The skin of *Ruvettus* is covered with prominent bony tubercles. Since this is the most striking morphological feature of the fish, it is unlikely that it would be omitted even in a brief description. *Ruvettus* is known for the strong purgative quality of its flesh (pharmaceutical properties have been studied by Macht and Barba-Gosé, 1931) and not for causing ciguatera. Vonfraenkel and Krick (1945) reported on the mass poisoning of 31 men by a 50 pound barracuda taken less than two years prior to the specimen of Cohen, Emert, and Goss, and at the same island, Saipan. The medical accounts of both of these papers are particularly valuable, because they show the variation in symptoms which may occur among ciguatera victims who have eaten the same

fish. The percentages of the principal symptoms are tabulated in both articles.

Poisonous fishes are associated with reefs. Fishes which occur over sand, mud, or turtle grass bottoms are not apt to be toxic. Of course, such fishes as certain jacks or barracuda may be caught away from reefs and still be poisonous. These predaceous fishes are inclined to rove, and the bulk of their prey could come from on or near reefs even though they are taken over sand.

Most of the poisonous fishes attain moderate or large size. Although fishing effort, in general, is directed towards the larger fishes and hence there is less opportunity for small fishes to poison people, it is nevertheless apparent that the larger species are consistently more dangerous. Within the genus *Lutjanus* in Tahiti, the species *L. bohar, L. monostigmus,* and *L. rivulatus,* all of which may exceed 400 mm in standard length, are the worst offenders. *L. vaigiensis* and *L. kasmira,* which rarely exceed 250 mm in standard length, are more frequently caught and much safer to eat. *Cephalopholis argus* has been cited as toxic, but the smaller groupers *C. urodelus* and *C. leopardus* are not considered dangerous.

Fishes which cause ciguatera are carnivores or feeders on detritus or benthic algae. None appear to be plankton-feeders as adults (poisoning from certain plankton-feeding clupeid fishes, as mentioned, appears to differ from ciguatera). In Tahiti the carangid fishes *Selar crumenophthalmus* and *Decapterus pinnulatus* are among the most abundant food fishes and are said to be universally nonpoisonous. They are open-water species, usually found near shore, and probably feed on zooplankton and the smaller nekton. In more western parts of Oceania, species of the plankton-feeding lutjanoid genera *Caesio* and *Pterocaesio* are common and are not suspected of causing ciguatera.

Among poisonous fishes which feed primarily on fishes and crustaceans, there appears to be a correlation between the amount of fish in the diet and the degree of toxicity, although the tendency for the larger fishes, in general, to be piscivorous is unquestionably a factor and difficult to assess. The common groupers and snappers of the Society Islands are arranged in approximate order of potential toxicity as follows: *Lutjanus monostigmus, Plectropomus leopardus, Variola louti, Cephalopholis argus, Epinephelus elongatus, Lutjanus vaigiensis, Lutjanus kasmira, Epinephelus hexagonatus,* and *Epinephelus merra.* The first three species appear to be piscivorous. About

three-fourths of the *C. argus* examined had eaten fish and the rest crustaceans. The next four species feed approximately half the time on fish and half on crustaceans. Two-thirds of the specimens of the least toxic species, *E. merra,* were found with crustaceans in their stomachs and most of the remaining ones with fish. For more detailed data on the food habits of these fishes, consult Randall and Brock (MS, see references). Wheeler (in Wheeler and Ommanney, 1953: 32-37) has pointed out that *Lutjanus bohar, Lethrinus miniatus, Variola louti, Plectropomus maculatus,* and *Aprion virescens* feed mainly on fish. These fishes, as previously discussed, may be strongly toxic. Some of the groups of fishes, such as the squirrelfishes (Holocentridae) and goatfishes (Mullidae) which are rarely implicated in ciguatera, seem to be primarily crustacean feeders. Hiatt and Strasburg (MS, see references) examined the stomach contents of ten species of holocentrids and five mullids from the Marshall Islands. Crustaceans predominated in the diet of these fishes. The largest of the squirrelfishes in Tahiti, *Holocentrus spinifer,* is said to cause illness occasionally. The stomachs of two of these were opened and found to contain fish.

In the West Indian region there is a suggestion that the same correlation holds. According to Gudger (1918), *Sphyraena barracuda* is "wholly a piscivore." *Seriola falcata, Scomberomorus* spp. and *Caranx* spp. are probably mainly fish eaters (Breder, 1948). Three specimens of *Mycteroperca venenosa* and two of *M. bonaci* obtained by the author contained only fish in their stomachs. Ten Nassau grouper (*Epinephelus striatus*), a species which may attain a length of about four feet and which is considered as always safe to eat, were found with food in their stomachs; five contained crustaceans, three octopus, one had eaten a parrotfish, and the last contained a single fish scale.

The carnivorous *Lachnolaimus maximus* in the Atlantic, *Monotaxis grandoculis* in the Indo-Pacific, and balistids are not piscivorous and yet may be poisonous. The first two appear to be primarily mollusk-feeders. Hiatt and Strasburg (MS, see references) found all of seven specimens of *M. grandoculis* which they examined to contain gastropods. Most of them also had small clams and about half of them crab fragments. A few had eaten heart urchins and polychaetes as well. Longley in Longley and Hildebrand (1941: 188) reported that *L. maximus* feeds on univalve and bivalve mollusks and the sea urchin *Echinometra.* Two collected by the author in the Bahamas contained

mostly small gastropods, along with a few pelecypods and small crabs. Triggerfishes seem to be very generalized in their food habits. The gut contents of eight specimens of *Balistapus undulatus* from the Gilbert Islands consisted of green algae, coralline red algae (*Jania*), fresh coral (*Acropora*), sea urchins, two crustaceans, one polychaete, and considerable bottom debris (Randall, 1955a: 224).

The food habits of surgeonfishes and knowledge of their relative toxicity may shed light on the nature of the organism producing ciguatera toxin. *Ctenochaetus striatus* (portrayed in color in Hiyama, 1943: Pl. 19, Fig. 52) is the most abundant reef fish of moderate size in the Society Islands and probably in many other island groups in the Pacific as well. It is the most commonly and most strongly toxic of all the acanthurids in the Societies. Specimens from the district of Hitiaa, Tahiti produced reactions in mongooses at the Hawaii Marine Laboratory which varied from no visible effect to lethal in two days (lethal dose, 48 grams). The small teeth of this species and others of this Indo-Pacific genus are numerous, elongate, and flexible in the jaws; in feeding they are whisked over the bottom and pick up primarily fine detrital material (Randall, 1955b). This surgeonfish would seem capable of grazing directly on only the most delicate of the algae. The stomach contents of 13 specimens of *C. striatus* from three of the Society Islands consisted primarily of fine inorganic sediment (mostly calcareous sand) and indefinable organic material; there were in addition numerous algal filaments and diatoms, occasional Foraminifera, and a few other minute invertebrates. Possibly coarser detached material is taken in than the small particle size of the gut contents would indicate, for the gizzard-like stomach of *Ctenochaetus* may serve to grind the ingested matter into finer form. Other surgeonfishes have rigid teeth and graze on attached algae; species of the genus *Naso* usually feed on coarse or leafy types, and those of the genus *Acanthurus,* which have close-set, denticulate teeth, on filamentous forms. Species of *Naso* are regarded as nonpoisonous in Tahiti. Hiyama (1943) spoke of *Naso brevirostris* as a useful food fish which is seen in large numbers in the markets. Species of *Acanthurus,* as previously mentioned, may be toxic, especially from areas where many other kinds of fishes are poisonous. At Maui a shipment of *Acanthurus triostegus* from the island of Palmyra, notorious for toxic fishes, was reported by Halstead and Bunker (1954a: 6) to have poisoned 24 persons "who developed the typical ichthyosarcotoxic syndrome" (although these authors failed to detect any toxin in extracts of muscle

scraps of the offending fish which were injected intraperitoneally into mice).

Some gastropods and echinoids (probably mainly herbivorous) are said to cause illness when eaten. Presumably this illness is like ciguatera, but confirmation on this point is needed. *Livona pica* was mentioned earlier in connection with the origin of the term ciguatera. In the Bahamas, old conch *(Strombus gigas)* are not eaten for fear of toxicity (possibly those causing poisoning are *Strombus samba).* In Moorea, Society Islands, the author was told that the sea urchin *Tripneustes gratilla,* the gonads of which are commonly eaten, is toxic in one part of the shore reef known to possess many poisonous fishes. One specimen of this urchin was shipped frozen to the Hawaii Marine Laboratory where it was fed to a mongoose; the latter died in two days after exhibiting ciguatera-like symptoms. Earle (1940) has reported on illness in Barbados from eating the ova of *Tripneustes esculentus* (=*T. ventricosus*), which is widely eaten in the West Indies; however, he attributed the symptoms, epigastric pain, nausea, diarrhea, urticaria, and migraine, to allergy.

Poisonous fishes are not universally found over a large area; their distribution is spotty. Even around a small island, fishes in one sector may be dangerous to eat while those from another are perfectly safe. Gudger (1930: 49) (after Mowbray, 1916) wrote, in reference to poisonous fishes at the Turk Islands, Bahamas, ". . . the fish from one side of the islands were much more dangerous than those on the other. This seems entirely preposterous, especially in the case of Grand Turk, an island only 1½ miles wide by 6 miles long, but such a statement is met with over and over again in articles on West Indian Ciguatera."

That poisonous fishes are found in one part of a reef and not another nearby suggests that reef fishes are, in general, nonmigratory. This is widely believed for most of the fishes; however, direct evidence is meager. Bardach and Menzel (1957: 130) demonstrated through tagging in Bermuda that most of the serranid fishes remain fairly stationary, although there may be considerable movement over a period of a year or two. Results of tagging *Acanthurus triostegus* in Hawaii (Randall, MS, see references) and *Epinephelus merra* in Moorea (Randall, MS) indicate that these two species are normally very limited in their movements. Some predaceous species which occur more in open-water, such as certain carangid fishes and barracuda, are not reef fishes in the strict sense, although frequently seen near or over reefs. Probably they are more migratory than the usual reef fishes;

however, they too seem to remain in the same general area.

A region once poisonous may lose its toxic fishes and vice versa. Banner and Randall (1952: 55) were informed by Gilbertese natives that no poisonous fishes existed at Onotoa Atoll in 1951, but one section of reef harbored toxic fishes for several years two years previously. The Line Islands in the Pacific will serve as a good example of a region with no history of poisonous fishes which suddenly developed a serious ciguatera problem (Ross, 1947; Halstead, 1956; P. D. F. Palmer, by correspondence).

According to Palmer, a resident of Fanning Island beginning in 1936 and present manager of Fanning Island Plantations Limited, fishes were first noticed to be poisonous at Kingman Reef in about 1935, "from the time the first aircraft tender anchored there to service the first trans-pacific planes." The establishment of shore bases at Palmyra in about 1937 coincided with the appearance of poisonous fishes at this atoll. At Christmas Island fish became toxic "shortly after the outbreak of the war and soon after U. S. troops moved in in force. Say 1942." Fanning Island's outbreak has been mentioned; it began in late 1945. "Any kind of fish poisoning—except by the tetraodon group—had been unknown for a hundred years!" Of Washington Island, Palmer writes, "Poisonous fish absolutely unknown to this day and for the past century." "The islets Jarvis, Malden, and Starbuck as far as is known are free of poisonous fishes and have always been, but this cannot be proved for certain as they have seldom been inhabited and, when so, only for brief periods by very small groups." Palmer states that the problem of poisonous fishes appears to have abated in recent years, although occasional toxic fish are still encountered. According to E. Forsythe, fishes caught off Fresh Creek, Andros Island, Bahamas, following a severe storm in 1908 caused numerous cases of ciguatera. The problem was of such magnitude that the area was closed to fishing. In several years it became apparent that the fishes were again safe to eat. Thompson (1940: 137-138) noted that fishes in a 200 meter sector of lagoon shore at Tokalau, Fiji became poisonous following a hurricane of 1929. Local native people believed that the fishes might have been poisoned by seaweed that grew after the storm.

In the Society Islands the places pointed out to the author as dangerous with respect to ciguatera are areas of slight or intermittent freshwater drainage. On Moorea the shore at the entrance to Papetoai Bay possesses poisonous fishes, as to a lesser degree does Tareu Pass

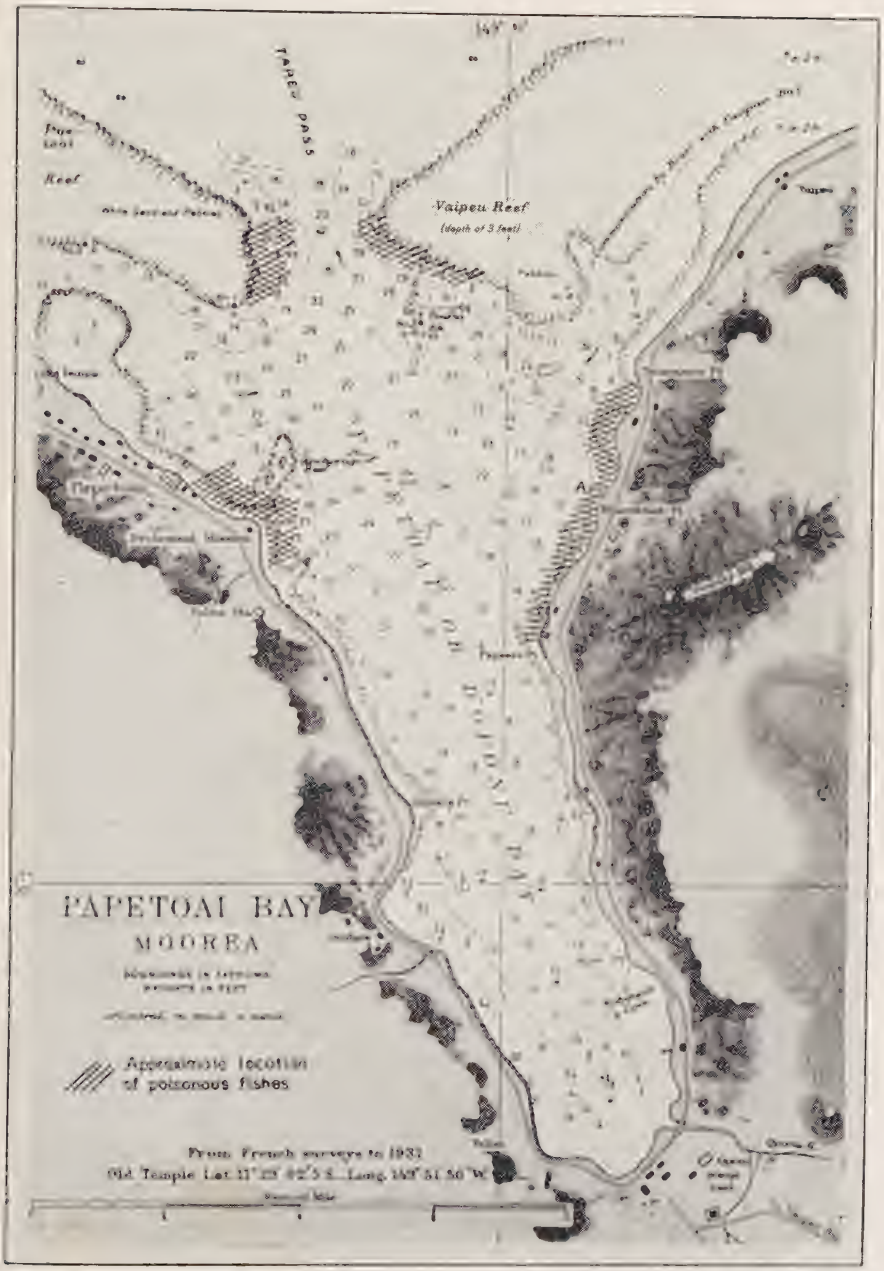


FIGURE 1. Distribution of poisonous fishes in Papetoai Bay, Moorea.

which leads to the bay (Fig. 1). The head of the bay into which a river flows is brackish and contains no toxic fishes. The barrier reef away from the pass and the shore reef away from the entrance are free of poisonous fishes; these areas are continuously flushed with clear water of the open sea which pours over the barrier reef from surf action. The most poisonous region in this part of Moorea is said to be about a 100 meter sector just to the east of the bay entrance (approximately at point A on Fig. 1). The bed of a small stream bisects this sector. Most of the time there is no obvious flow, and the stream bed contains stagnant pools of freshwater. At time of heavy rains (which are not uncommon in Moorea) a considerable outflow of freshwater from the land occurs at this and other drainage sites. When heavy rainfall is of long duration, water of salinity decidedly lower than usual is found at the entrance to the bay and in the pass.

The information on the boundaries of the toxic areas was supplied by local Tahitian people. As would be expected, they were not always in agreement as to the exact location of these boundaries. The opinions of these people were in part confirmed by finding poisonous fishes in the bad areas and interviewing persons poisoned from eating fishes taken from these sectors. Specimens of *Lutjanus monostigmus* and *Cephalopholia argus* from the vicinity of point A which were sent frozen to the Hawaii Marine Laboratory were poisonous to mongooses, as was the echinoid *Tripneustes gratilla* mentioned previously. While the author was in Moorea, two Tahitian families were poisoned by eating the flesh of a 900 mm *Plectopomus leopardus* taken by him on the east side of the bay near the boundary zone. Shortly thereafter a man and his wife contracted ciguatera after eating the liver of a very large *Caranx* caught just west of Tareu Pass. The man's illness was more severe than his wife's (he lost consciousness for several hours). He had been mildly poisoned only a week before by eating a triggerfish taken near Faatoai. The hotel proprietor in Faatoai was poisoned by mullet *(Crenimugil crenilabus)* taken at the entrance to the bay on the west side; the residual tingling sensations in his extremities lasted about four months. The local school teacher speared a large *Lutjanus monostigmus* at about the same place which was eaten by his pregnant wife and child. The wife became so ill with ciguatera that she aborted and nearly lost her life. She was bedridden three weeks and stated that she could not eat fish for eight months without reappearance of sensations in her hands or feet on contact with cold water which she described as "like ants crawling." The child vomited the piece of

snapper which he had eaten and was only slightly sick.

In the district of Mataiea on the southern shore of Tahiti the Vairaharaha River flows into the sea in line with Rautirare Pass, and the adjacent Potiai River into Aifa Pass. Between these two passes is a section of barrier reef which is devoid of poisonous fishes. Toxic fishes are found on the east side of Rautirare Pass and the west side of Aifa Pass. According to Jacques Drollet, formerly a school teacher in the district, the circulation of the waters of this region is as indicated in Figure 2. The east side of Rautirare Pass and the west side of Aifa Pass are occasionally turbid and roily with freshwater whereas the other sides of these passes are relatively clear. The current created from the powerful surge over the center of the intermediate barrier reef apparently keeps the inner sides of the passes nearly free of run-off from the land.

In Tahiti the western (lee) districts such as Punaauia are relatively free of poisonous fishes while the wetter eastern districts like Hitiaa have many more poisonous fishes. The worst sector of Hitiaa is noteworthy for containing the mouth of a stream.

According to Mrs. J. Jacquemin, the only poisonous fishes at Makatea (not an atoll) in the Tuamotu Archipelago occur at the mouth of the one small stream on the island. Flow from this stream is probably not continuous.

On Tikahau Atoll in the Tuamotus the grouper which poisoned the author and two associates was speared in a basin about one-half mile south of the pass. This basin is nearly cut off from the rest of the lagoon by a semicircle of coral reef, and the water in the basin was more turbid and a different color of green (presumably from phytoplankton) than the rest of the lagoon. It is inferred that drainage from the adjacent islet is not readily flushed from this region as it is elsewhere in the lagoon.

The great majority of the records of poisonous fishes are from islands. Although there is undoubtedly more fish eaten per capita in insular areas than continental regions, it nevertheless seems apparent that this alone cannot account for the big discrepancy. The island environment seems more conducive to ciguatera. One factor may be the greater percentage of reef areas.

Hypothesis for Cause of Ciguatera

1. Fishes become poisonous because of some factor in their environment. If all fish of one species were toxic wherever found, either

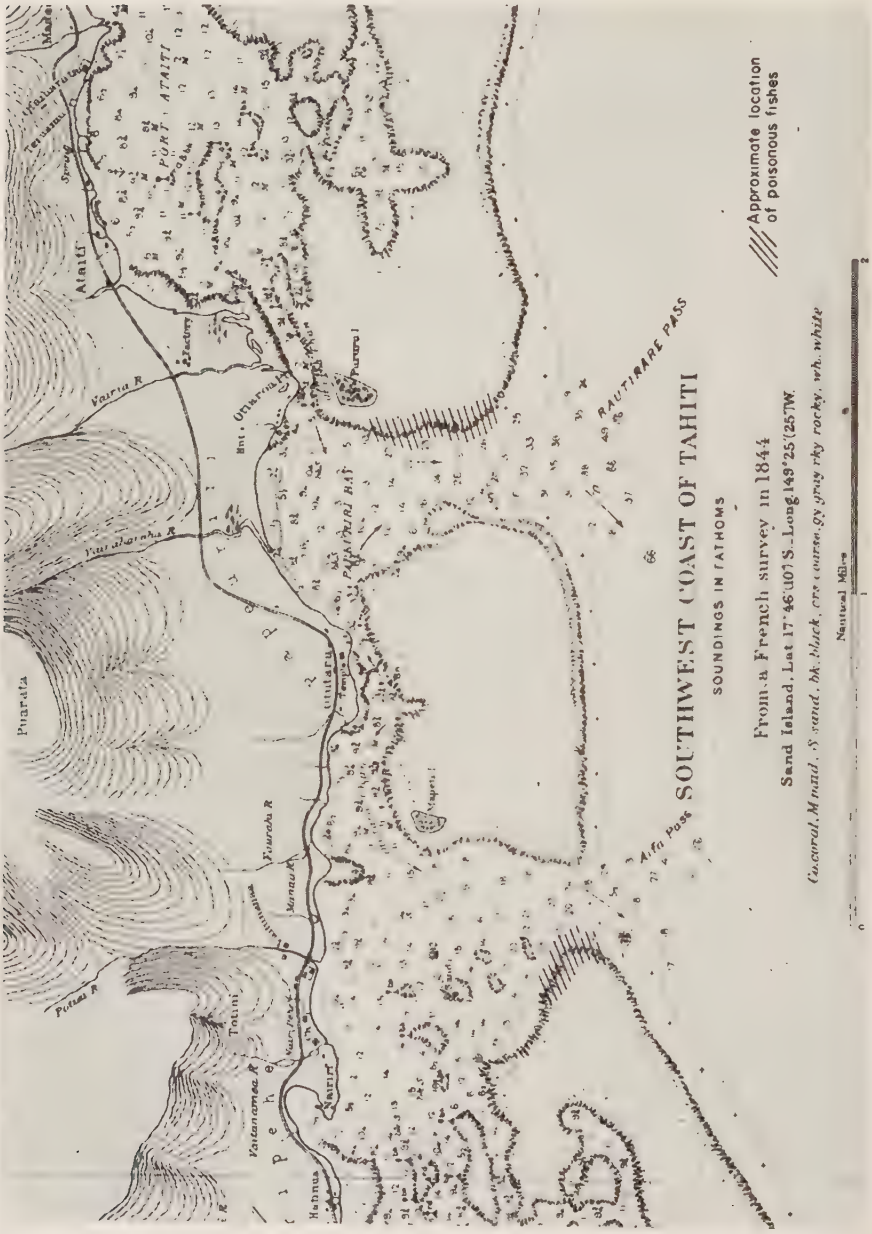


FIGURE 2. Distribution of poisonous fishes in Aifa and Rautirare Passes, Southwest coast of Tahiti.

continuously or at a certain season, then the toxin could be expected to be endogenous; but when fish in a restricted area are toxic and those of the same species and same size in adjacent areas are not, then the toxin must arise from the environment.

2. The toxicity of fishes is associated with their food supply. Even in the most poisonous areas, only certain of the fishes cause illness when eaten; therefore a differential source of toxin must be found. The most logical would be the food of the fishes, which differs from species to species.

3. The basic poisonous organism is benthic. Plankton-feeding fishes and invertebrates apparently do not cause ciguatera. Regions of reef or shore may harbor poisonous fishes, while on adjacent areas the same species may be safe for consumption. Yet both areas may be washed by seas containing essentially the same plankton.

4. Since obligately herbivorous fishes and detritus-feeding fishes may be poisonous, the toxic organism would most likely be an alga, a fungus, a protozoan, or a bacterium. A herbivorous fish might ingest any of the latter three categories with its algal food, and the detritus-feeder could obtain any of these incidentally in its feeding.

If an alga, it must be fine, because certain potentially toxic surgeonfishes such as *Acanthurus triostegus* cannot feed on coarse types.

5. Of the algae, blue-greens (Cyanophyta) would seem to be the most probable source for ciguatera toxin. Certain planktonic blue-greens are notorious for fouling lakes and on occasions killing domestic animals. Ingram (1953) listed 36 references to toxic blue-green algae from freshwater. Steyn (1945) reported that fresh, growing *Microcystis* is toxic, and when it dies and decomposes, the toxin passes into the water. When water containing the alga is boiled, the toxicity is not reduced. Vinberg (1954) believes that Haff disease, an illness (with symptoms which differ from ciguatera) which occurs about 18 hours after eating certain fishes from the freshwater bay of Frisches Haff off the Baltic Sea and lakes in Sweden and Russia, is due to a toxin derived from planktonic blue-green algae and carried through food chains to the food fishes. Luxurious blooms of blue-green algae were observed in years when outbreaks occurred.

Additional evidence is provided by the food habits of surgeonfishes. In preference feeding experiments with different kinds of algae, *Acanthurus triostegus* fed avidly on certain green and red algae but avoided

all blue-greens (five species tested) (Randall, MS, see references). Blue-greens may be found in the stomachs, however, but always mixed with other algae (Dawson, Aleem, and Halstead, 1955: Table 1). As previously discussed, species of *Naso* tend to feed on coarse algae; these fishes are the least toxic of the tropical surgeonfishes. The detritus-feeding *Ctenochaetus* has the worst reputation. Species of *Acanthurus* are usually poisonous only in particularly bad sectors. Of the three genera, *Naso* would be least likely to ingest a toxic blue-green (unless an epiphyte); *Acanthurus* could avoid patches of the hypothetical blue-green while feeding, but if the alga were inextricably mixed as one of the dominant turf algae, as it might be in a poisonous area, some would be taken in. *Ctenochaetus* is the least discriminating in its feeding and could consistently obtain the alga as part of the detrital mass. Similarly, herbivorous gastropods and echinoids might not be expected to be very selective in their feeding.

6. The most poisonous fishes are the large predaceous species, especially those that feed on fishes. Frequently these are the only fishes in an area which will cause ciguatera. In view of Takata's finding that the toxin dissipates slowly, if at all, in poisonous fishes, it would seem that they would accumulate the toxin; thus the older, hence larger, fish would be the most dangerous.

At present the only way to detect toxicity of an organism is to feed it to a susceptible animal and observe the outcome. If there are no symptoms of ciguatera, the fish is assumed to be nonpoisonous. It still may contain toxin, however, at the subsymptomatic level. If a person recovering from ciguatera eats a reef fish which causes no distress in other persons, he may experience a return or intensification of neurotoxic symptoms. The fish evidently contains enough toxin to raise that already present in the system of this person to the threshold level, but not enough to make persons ill who have not eaten poisonous fish shortly before.

In an area where only the large carnivorous fishes are toxic, any one fish or invertebrate comprising the prey of such fishes would not have enough toxin to elicit symptoms if eaten by man. With the ingestion of each fish or invertebrate containing some toxin, the amount within the predator is raised. When a predator eats a herbivorous or detritus-feeding fish or invertebrate or a carnivore that preys on such forms, he acquires in one short period the toxin accumulated by the animal over its lifetime, assuming complete assimilation. It would seem possible

that all animals on a toxic reef whose food leads back through chains to benthic algae or detritus could have some toxin in them, albeit in minute amounts in many.

The apparent tendency for piscivorous fishes to be more toxic than crustacean-feeders may be due to fishes obtaining a higher percentage of the available algal or detrital mass than crustacea.

Another important factor which contributes to the greater toxicity of larger piscivorous fish is the relatively large size of most herbivorous and detritus-feeding types. The most abundant plant-feeding fishes in tropic seas are the acanthurids, along with teuthidids in much of the Indo-Pacific. Kyphosid fishes feed on algae and are common in some places. The omnipresent Scaridae (parrotfishes) represent a group which appears to be specialized for feeding on boring algae but also ingest some surface types and detritus. Pomacentrid and chaetodont fishes are common omnivorous groups. With the exception of the smaller Pomacentridae, all of these fishes are moderate in size, and all but the parrotfishes are high-bodied. Only the larger predaceous fishes could engulf such species as adults.

7. The organisms producing ciguatera toxin may be one of the first growing on new or denuded surfaces in tropic seas in normal ecological succession. With this in mind many of the peculiarities of the distribution of poisonous fishes become explicable.

The history of poisonous fishes in the Line Islands led the author into this concept. Suspecting from the association of toxic fish in the Society Islands with regions of slight freshwater drainage (but not brackish areas) that there might be a connection between nutrients in the run-off from the land and blue-green algal growth (Edmondson, Anderson and Peterson, 1956, and other authors have demonstrated the predilection of certain blue-greens for eutrophic conditions), a letter was written to P. D. F. Palmer on Fanning Island to inquire of the disposition of sewage of the troops stationed there and on Christmas Island during World War II. Mr. Palmer replied that toxic areas had not been ones of sewage disposal. "The deadly areas that are still occasionally toxic today are only where the ships anchored." The worst area at Fanning was English Harbour. Here also is where Ross indicated that war materials were dumped. At each island in the Line Islands where poisonous fishes suddenly appeared there is record about the same time of the anchoring of large ships.

Palmer noted damage to the coral in the anchorage in English Har-

bour which "can be clearly seen from the surface as large white scars." This was "caused by the crashing of innumerable heavy ships anchors and ploughing caused by dragging anchor cables." Thus, new substratum became available for marine growth. Also, much debris, such as bottles and tin cans, is thrown overboard from ships at anchor which accumulates beneath the ships and provides new surfaces.

It is significant that Washington, which has never had any poisonous fishes, was not visited by military vessels during the war. Palmer further refuted the idea that there may be a direct effect from freshwater drainage by pointing out that Kingman Reef has no land area, and "Washington, the only permanently occupied, nontoxic atoll, is the only one with a freshwater lake and steady head of freshwater flowing to seaward all the year around from the heavy rainfall."

The association of poisonous fishes with freshwater drainage in the Society Islands can be explained in terms of new substratum. There are no poisonous fishes at the head of Papetoai Bay, Moorea, where a river empties continuously. The toxic fishes are found at the entrance to the bay and Tareu Pass, areas of seawater salinity except at times of long, heavy rain. Similarly the stream bed opening into the center of a 100 meter section of shore reef said to be the most dangerous sector in the vicinity becomes a route for freshwater flow only during and just following precipitation. It is possible that the normal marine flora is killed by the outflow of turbid freshwater, thus exposing surfaces for new algal growth after the freshwater is swept away. The same sequence of events may have led to the outbreak of ciguatera off Fresh Creek, Andros, after the severe storm of 1908.

It is possible that new substratum could be provided by wave action during storms. This might be caused by scouring, by the dislodging of pieces of a reef, or by a shifting of sand to expose previously covered sections of reef. The latter situation need not require heavy wave action. A slight alteration in hydrographic conditions could result in the exposure of solid surfaces by shifting of sand.

If a steep reef front is exposed to the attrition of waves and boring organisms, pieces could fall from it and roll into deeper water, as could overhanging parts of islands at the water's edge, (often seen in the Bahamas). The north (actually west) side of Grand Turk in the Bahamas said by Mowbray (1916) to contain many poisonous fishes is precipitous. Brown (1945: 36) has written, "In most cases the windward or eastern steep-to exposed areas have a bad reputation and the leeward or shallow bank areas are regarded as safe, for the same

species of fish at the same time. A similar distribution of poisonous fish is known to fishermen in the Bahamas (*e.g.*, at Ragged Island)."

New surfaces are constantly being formed on reefs by the feeding of grazing animals, especially those like parrotfishes which can scrape sections completely bare. In this case, the effect is generally too diffuse to bring about a concentration of new growth, but it may be pointed out that each isolated little scraped area becomes a focus for settling forms; collectively these little areas maintain those organisms in the region which appear early in ecological succession, only to give way to subsequent sessile life.

Wrecks of ships have frequently been given as the location of poisonous fishes. It may be said that a wreck forms such a well-known land mark that it could gain a bad reputation from an occasional poisonous fish that another area might not; however, this belief is met with so frequently that there would seem to be some real basis for it. Here, again, a new surface becomes available for population by benthic marine organisms. Furthermore, in the case of a wreck, new surfaces may appear if it breaks up.

As previously mentioned, copper has been linked with ciguatera because of the occurrence of poisonous fishes around copper-bottomed ships, both as wrecks and intact sailing vessels. In the latter case, during long voyages, the ships acquired heavy marine growth on their hulls. Also, islands with copper mines, such as Virgin Gorda, have been associated with a high incidence of poisonous fishes, and the toxicity attributed to possible copper banks offshore. If such associations are valid, and they appear to be, then they too may conform to the concept of a new substratum in the sea. While in the Virgin Islands, Conrad Limbaugh was informed that rubble from copper mines was often dumped in the sea.

The mere presence of a new surface in tropic seas does not mean that ciguatera will surely follow. Clearly, other environmental conditions must also be satisfied. Why, for example, have the populous Hawaiian Islands been spared serious outbreaks of ciguatera? There have been outbreaks at Johnston Island (Halstead and Bunker, 1954b) and Midway (Lee and Pang, 1944), both very similar in marine flora and fauna to the Hawaiian Islands proper. These are correlated in time with military activities and may have been caused by a disrupting of the reefs or dumping of debris as postulated for the Line Islands. During war time increased shipping to Oahu would mean only more ships in mud-bottomed Pearl Harbor or Honolulu Harbor

where no amount of disturbance would be expected to create poisonous fishes. Nevertheless, there would seem to have been enough alteration of certain reef areas by dredging, blasting, etc., around the principal Hawaiian Islands (and by lava flows on the island of Hawaii) to expect poisonous fishes to occur.

It may be worth while to note that Johnston and Midway differ from the larger Hawaiian Islands in possessing lagoons. Cohen, Emert, and Goss (1946) commented on the occurrence of ciguatera at atolls of the Marshalls and Gilberts and its apparent absence at the nearby high islands of Ocean and Nauru which lack lagoons. It is not inferred that lagoons or lagoon-like conditions are necessary for the growth of the organism(s) producing ciguatera toxin, for there are exceptions, such as Makatea, but areas with bodies of water set off from the open sea by reefs may be more prone to contain poisonous fishes.

A reef organism may be abundant at one island and not at a second which outwardly may appear identical in its marine environment. The coral reef community is probably the most complex in the sea, and a very delicate balance must exist among organisms in competition, such that a very subtle change in the environment can result in the proliferation of one at the expense of the others. The toxic organism may be but one of a group of competing forms.

Recommendations for Future Research

Research on ciguatera should be directed toward the attainment of the following three goals: (1) the identification of the organism producing the toxin, and the analysis of the environment in which it flourishes; (2) the discovery of a simple test for determining whether a fish is poisonous or not; and, (3) the development of an antitoxin.

As far as the author knows, no work is being done to achieve the last objective. A. H. Banner is heading research designed to find a sensitive field test for detection of ciguatera toxin in marine organisms. The achievement of this second goal would greatly facilitate attainment of the first.

It is recommended that the hypothesis presented in this paper to explain the cause of ciguatera be put to test. Such work should be undertaken in an area where the problem of fish poisoning is acute. It should take two approaches, the investigation of a small sector such as the one in Moorea with a long history of strongly toxic fishes, and the creation of an extensive area of new surface in the sea in a region of reef which would seem to have the potentiality of becoming toxic

but which is demonstrated to possess no poisonous fishes by feeding numerous large specimens to suitable experimental animals.

A study should be made of the ecological succession of organisms appearing on the new surface. If the region is subject to even slight seasonal variation in sea temperature or other hydrographic factors, it might be advisable to initiate the study several times during the year.

The new surface should be non-corrosive, durable, and permit easy removal of the marine growth. It should also be attractive to reef fishes and invertebrates of variable size by providing suitable shelter.

If an organism appeared on the surface in profusion and essentially in pure stands, it could be scraped off, identified, extracted by the method of Banner and Boroughs, mixed with an appropriate food, and fed to an experimental animal such as a mongoose (or tested directly if a better method of determining the toxin is devised).

If the toxin is in low concentration in the basic organism, it might not be detectable by feeding extracts of even large amounts to experimental animals. Under the circumstances the toxin would then be sought in marine animals observed to feed on benthic growth in the area.

Much more fundamental research is needed on the food habits of reef animals before those animals which first acquire the toxin can be determined and the manner in which the toxin is transported through food chains to the larger food fishes is ascertained. The study of the age and growth and movements of reef organisms would also be significant to the comprehension of ciguatera.

REFERENCES

Arcisz, W.
1950. Ciguatera: tropical fish poisoning. Spec. sci. Rep. U. S. Fish Wildl. Serv. No. 27. 23 pp.

Banner, A. H. and H. Boroughs
MS. Preliminary observations on the toxins of poisonous fishes. Submitted to Proc. Soc. Exper. Biol. and Med.

Banner, A. H. and J. E. Randall
1952. Preliminary report on marine biology study of Onotoa Atoll, Gilbert Islands. Atoll Res. Bull. No. 13. 62 pp.

Bardach, J. E. and D. W. Menzel
1957. Field and laboratory observations on the growth of some Bermuda reef fisheries. Proc. Gulf Carib. Fish. Inst. 9th Ann. Ses. 1956: 106-112, 129-131.

Belotte, J.
1955. Les poissons "empoisonnés" du lagon Tahitien. Méd. trop., *15:* 232-236.

Breder, C. M., Jr.
1948. Field book of marine fishes of the Atlantic Coast from Labrador to Texas. xxxvii + 332 pp. G. P. Putnam's Sons, New York.

Brown, H. H.
1945. The fisheries of the Windward and Leeward Islands. Develop. & Welfare in West Indies Bull. No. 20. 97 pp.

Buddle, R.
1930. Some common poisonous fishes found in Singapore waters. J. Roy. Nav. Med. Serv., *16:* 102-111.

Cohen, S. C., J. T. Emert and C. C. Goss
1946. Poisoning by barracuda-like fish in the Marianas. U. S. Navy Med. Bull., *46* (2): 311-317.

Dawson, E. Y., A. A. Aleem and B. W. Halstead
1955. Marine algae from Palmyra Island with special reference to the feeding habits and toxicology of reef fishes. Occ. Pap. Allan Hancock Fdn. No. 17. 39 pp.

de Sylva, D.
1956. Poisoning by barracuda and other fishes. Mar. Lab. Univ. Miami. Spec. Serv. Bull. No. 13. 9 pp.

Earle, K. V.
1940. Pathological effects of two West Indian echinoderms. Trans. Roy. Soc. Trop. Med. Hyg., *33* (4): 447-457.

Edmondson, W. T., G. C. Anderson and D. R. Peterson
1956. Artificial eutrophication of Lake Washington. Limnol. Oceanogr., *1* (1): 47-53.

Fish, C. J. and M. C. Cobb
1954. Noxious marine animals of the Central and Western Pacific Ocean. Res. Rep. U. S. Fish Wildl. Serv. 36. iii + 45 pp.

Fitch, J. E.
1952. Toxicity and taxonomic notes on the squaretail, *Tetragonurus cuvieri.* Calif. Fish Game, *38* (2): 251-252.

Fowler, H. W.
1928. The fishes of Oceania. Mem. Bishop Mus., *10:* iii + 540 pp.

Goode, G. B. and T. H. Bean
1895. Oceanic ichthyology. Spec. Bull. U. S. Nat. Mus., xxxv + 553 pp.

Gudger, E. W.
1918. *Sphyraena barracuda;* its morphology, habits, and history. Pap. Tortugas Lab., *12* (4): 53-108. (Publ. Carnegie Inst. Wash. No. 252.)
1930. Poisonous fishes and fish poisonings with special reference to Ciguatera in the West Indies. Amer. J. Trop. Med., *10* (1): 43-55.

Habekost, R. C., I. M. Fraser and B. W. Halstead
1955. Toxicology. —Observations on toxic marine algae. J. Wash. Acad. Sci., *45* (4): 101-103.

Halstead, B. W.
1954. A note regarding the toxicity of the fishes of the skipjack family, Katsuwonidae. Calif. Fish Game, *40* (1): 61-63.
1956. Current status of research on Pacific poisonous fishes and ichthyosarcotoxism. Venoms. Amer. Assoc. Adv. Sci., 29-32.

Halstead, B. W. and N. C. Bunker
1954a. A survey of the poisonous fishes of the Phoenix Islands. Copeia, 1954, (1): 1-11.

1954b. A survey of the poisonous fishes of Johnston Island. Zoologica, *39* (2): 61-77.

Halstead, B. W. and W. M. Lively
1954. Poisonous fishes and ichthyosarcotoxism. U. S. Forces Med. J., *5* (2): 157-175.

Harry, R. R.
1953. Ichthyological field data of Raroia Atoll, Tuamotu Archipelago. Atoll Res. Bull. No. 18. 190 pp.

Hashimoto, Y.
1956. A note on the poison of a barracuda *Sphyraena picuda* Bloch and Schneider. Bull. Jap. Soc. Sci. Fish., *21* (11): 1153-1157.

Hiatt, R. W. and D. W. Strasburg
MS Biotic interaction on coral reefs of the Mashall Islands with emphasis on the fish fauna. Submitted to Ecol. Monogr.

Hiyama, Y.
1943. Report of an investigation of poisonous fishes of the South Seas. 137 pp. Nissan Fish. Exp. Stat., Japan. (Translation to English by W. G. Van Campen in Spec. sci. Rep. U. S. Fish Wildl. Serv. No. 25. 1950.)

Hughes, E. O., P. R. Gorham and A. Zehnder
1958. Toxicity of a unialgal culture of *Microcystis aeruginosa*. Can. J. Microbiol., *4* (3): 225-236.

Hutner, S. H. and J. J. A. McLaughlin
1958. Poisonous tides. Sci. Amer., *199* (2): 92-98.

Ingram, W. M., *et al.*
1953. Handbook of selected biological references on water pollution control, sewage treatment, water treatment. Publ. Hlth. Serv. Publ. Wash. No. 214. x + 66 pp.

Jordan, D. S.
1929. Poisonous fishes in Samoa. Amer. Nat., *63:* 382-384.

Khlentzos, C. T.
1950. Seventeen cases of poisoning due to ingestion of an eel, *Gymnothorax flavimarginatus*. Amer. J. Trop. Med., *30* (5): 785-793.

Lee, R. K. C. and H. Q. Pang
1944. An outbreak of fish poisoning in Honolulu, Hawaii. Hawaii Med. J., *4*(1): 129-132.

Longley, W. H. and S. F. Hildebrand
1941. Systematic catalogue of the fishes of Tortugas, Florida. Pap. Tortugas Lab., *34:* xiii + 331 pp. (Publ. Carnegie Inst. Wash. 535.)

Macht, D. I. and J. Barba-Gosé
1931. Pharmacology of Ruvettus Pretiosus, or "Caster-oil Fish." Proc. Soc. Exp. Biol. Med., *28*(4): 772-774.

Mills, A. R.
1956. Poisonous fish in the South Pacific. J. Trop. Med. (Hyg.), *59*(5): 99-103.

Mohler, W. A.
1941. Een blauwwieren-phenomeen aan het strand van Balikpapan. Natuurw. Tijdschr. Ned. Indie, *101*(3). (Reference from Fish and Cobb, 1954.)

Mowbray, L. L.
1916. Fish poisoning (Ichthyotoxisimus). Bull. N. Y. Zool. Soc., *19*(6): 1422-1423.

PHISALIX, M.
1922. Animaux venimeux et venins. Vol. 1, xxv + 656 pp. Masson & Cie, Paris.

PILLSBURY, R. W.
1957. Avoidance of poisonous eggs of the marine fish *Scorpaenichthys marmoratus* by predators. Copeia 1957, (3): 251-252.

POEY, F.
1866. Ciguatera; memoria sobre la enfermedad ocasionada por los peces venenosos. Repertorio Fisico-Natural de la isla de Cuba. 39 pp.

RALLS, R. J. AND B. W. HALSTEAD
1955. Moray eel poisoning and a preliminary report on the action of the toxin. Amer. J. Trop. Med. Hyg., *4*(1): 136-140.

RANDALL, J. E.
1955a. Fishes of the Gilbert Islands. Atoll Res. Bull. No. 47. 243 pp.
1955b. A revision of the surgeon fish genus *Ctenochaetus*, family Acanthuridae, with descriptions of five new species. Zoologica, *40*(4): 149-166.
MS. A contribution to the biology of the convict surgeon fish or manini (*Acanthurus triostegus*) of the Hawaiian Islands. Submitted to Ecol. Monogr.

RANDALL, J. E. AND V. E. BROCK
MS. Notes on the ecology of the common epinepheline and lutjanid fishes of the Society Islands, with emphasis on food habits. Submitted to J. Wildl. Manage.

RISSO, A.
1810. Ichthyologie de Nice . . . 388 pp. Paris.

ROSS, S. G.
1947. Preliminary report on fish poisoning at Fanning Island. Med. J. Aust., *2*(21): 617-621.

SCHULTZ, L. P., *et al*
1953. Fishes of the Marshall and Marianas Islands. Bull. U. S. Nat. Mus. 202, vol. 1, xxxii + 685 pp.

SCHULTZ, L. P. AND E. M. STERN
1948. The ways of fishes. xii + 264 pp. D. van Nostrand Co., New York.

STEPHENSON, J.
1838. Medical zoology, and mineralogy. vi + 350 pp. John Churchill, London.

STEYN, D. G.
1945. Poisoning of animals and human beings by algae. S. Afr. J. Sci. *41:* 243-244.

STRONG, R. P.
1944. Stitt's diagnosis, prevention and treatment of tropical diseases. 7th edition, vol. 2. vii + 872-1747 + xl pp. Blakiston, Philadelphia.

THOMPSON, L.
1940. Southern Lau, Fiji: an ethnography. Bull. Bishop Mus., 162. iv + 228 pp.

THOMSON, W. K., A. C. LAING AND G. A. GRANT
1957. Toxic algae IV. Isolation of toxic bacterial contaminants. Def. Res. Kingston Lab. (Canada) Rep. 51. iii + 7 pp.

Titcomb, M.
1952. Native use of fish in Hawaii. 162 pp. Avery Press Limited, New Plymouth, N. Z.

Veenhuyzen, J. C.
1879. Communication on: *Trichodesmium erythraeum*. Natuurk. Tijdschr. Ned. Indie, *38:* 150-151. (Reference from Fish and Cobb, 1954.)

Vinberg, G. G.
1954. Toksicheskii fitoplankton (Toxic phytoplankton). Uspekhi Sovremennoi Biologii [Progress of Contemporary Biology (USSR)], *38:* 216-226. (Translation to English by G. Belkov in Nat. Res. Coun. Canada Tech. Transl. 549. 1955.)

Vonfraenkel, P. H. and E. S. Krick
1945. Fish poisoning by barracuda in the Marianas. Bull. U. S. Nav. Med., *44*(2): 427-431.

Wheeler, J. F. G. and F. D. Ommanney
1953. Report on the Mauritius-Seychelles Fisheries Survey 1948-1949. Colon. Off. Fish. Publ. *1*(3): 145 pp.

Whitley, G. P.
1943. Poisonous and harmful fishes. Coun. Sci. Indust. Res. Bull. No. 159, Melbourne. 28 pp.

Whitley, G. P. and B. W. Halstead
1955. An annotated bibliography of the poisonous and venomous fishes of Australia. Rec. Aust. Mus., *23*(5): 211-227.

Yudkin, W. H.
1944. Tetrodon poisoning. Bull. Bingham Oceanogr. Coll., *9*(1): 1-18.

THE PLANKTONIC LARVAE OF *POLYDORA WEBSTERI* HARTMAN (ANNELIDA, POLYCHAETA) AND THEIR SETTLING ON OYSTERS

SEWELL H. HOPKINS
A & M College of Texas, College Station

ABSTRACT

Larvae that emerged spontaneously from tubes of the "mudworm" *Polydora websteri* Hartman at 24°C had three setiferous segments like the newly-emerged larvae of *P. ciliata* (Johnston), but differed from the latter in ciliation, pigmentation, and number of setae. Planktonic *Polydora* larvae, presumed to be *P. websteri,* were found in Louisiana waters throughout the year. The largest larvae in plankton and the smallest worms on oysters had seventeen segments; this is assumed to be the stage at which settling occurs. Young *P. websteri* settle on the outside surfaces of oysters, or on the margins of the shells, and begin cutting grooves, which later become pits containing horseshoe-shaped burrows encased in mucus-cemented mud and continued on the outside by a pair of mud tubes projecting from the surface of the shell.

INTRODUCTION

Several species of "mudworms" of the genus *Polydora* Bosc, 1802, commonly live in galleries excavated in mollusk shells and have been accused of damaging or even killing oysters. Among these are *P. hoplura* (Claparède, 1869) in Europe (Carazzi, 1893; Korringa, 1951) and *P. ciliata* (Johnston, 1838) in Europe (Leloup, 1937) and in Australia (Whitelegge, 1890). *Polydora ligni* Webster, 1880, which builds mud tubes on the outside of oyster shells but supposedly does not excavate galleries in the shell, has been charged with oyster mortality in New Jersey by Nelson and Stauber (1940). These three are old and well-known species. *Polydora caeca* Webster, 1879 (not Oersted, 1843) was confused with *P. ciliata* until it was re-described and named *Polydora websteri* by Olga Hartman in Loosanoff and Engle (1943).

Lunz (1940, 1941) first drew attention to *P. websteri* (then called *P. ciliata)* as an enemy of oyster culture. It is now known to be associated with the commercial oyster, *Crassostrea virginica* (Gmelin), from Connecticut to Texas. Mackin and Cauthron (1952) showed that Louisiana oysters have especially heavy infestations of *Polydora websteri,* which cause damage to both shells and meats and may be a con-

tributing cause of mortality. Owen (1957) found a positive correlation between *Polydora* infestation and oyster mortality in the field, but could not substantiate this by controlled experiments and therefore concluded that *P. websteri* in Louisiana is not "the cause of mortality" but "contributes a part toward the formation of a poor environment and in most cases is indicative of such an environment." Owen apparently was not aware of the previous report by Mackin and Cauthron.

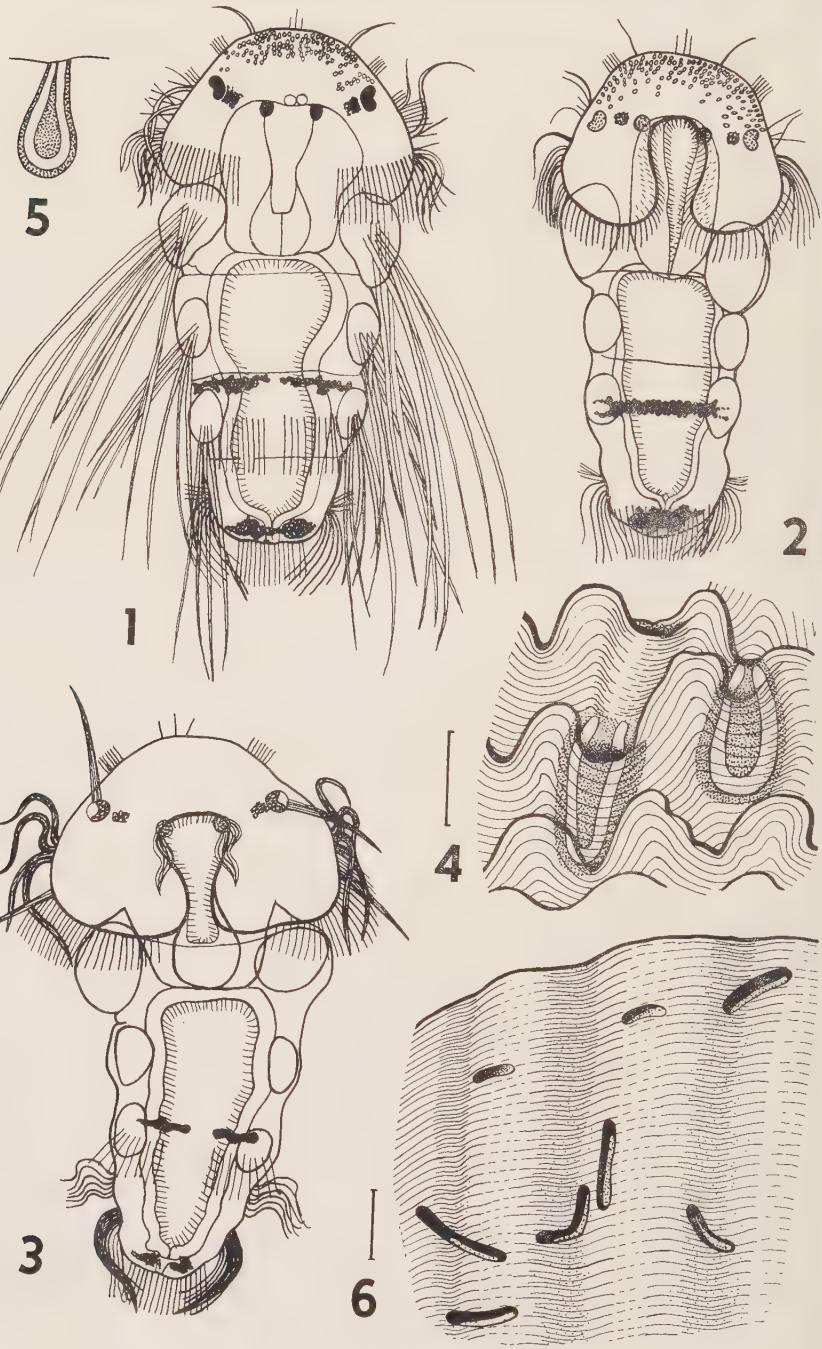
Larval stages of European species of *Polydora* have been described by many workers. The most important publications are those of Leschke (1903) and Wilson (1928), who first reared larvae in the laboratory, the monograph on the family Spionidae by Soederstrom (1920), and the recent review of Swedish species by Hannerz (1956). Very little has been published on the larval forms of American species of *Polydora*. Hartman (1941) described larvae of a few species on the Pacific coast.

The writer, working with Dr. J. G. Mackin, was able to make some observations on the larvae of *Polydora websteri* during a few hours salvaged from administration of an oyster mortality investigation. This was the Texas A & M Research Foundation's Project 9, which was supported by funds from The Texas Company, Humble Oil & Refining Company, The California Company, Phillips Petroleum Company, Shell Oil Company, and Tidewater Oil Company. Most of the work was done in the Louisiana State University Marine Laboratory at Grand Isle, Louisiana.

The Early Larvae of *Polydora websteri*

On November 17, 1947, an oyster from Bay Chene Fleur was being studied in a fingerbowl of sea water under a binocular dissecting microscope. A gooseneck lamp was bent down close over the oyster, providing considerable warmth as well as light. Suddenly streams of small annelid larvae began to shoot out of the numerous mud tubes of *Polydora websteri* around the margins of the oyster shell. Notes made at the time describe the fast-swimming larvae as "looking like a flight of rockets." Larvae continued to shoot out of the tubes in large numbers and swim toward the light, collecting in a dense swarm on the side of the dish nearest the lamp. The temperature of the water in the fingerbowl was 24°C.

Hundreds of *Polydora* larvae were drawn up into medicine droppers and transferred to separate dishes, and to slides, for further obser-


5
1
2
4
3
6

vation. Many were studied alive with and without intravital staining by neutral red. Camera lucida drawings of newly-emerged larvae are presented in Figures 1 and 2. All larvae seemed to be in exactly the same stage at the time of emergence. They were approximately 0.35-0.40 mm long and had three seta-bearing segments. On each side there were nine to thirteen setae on the first segment, five or six on the second, and four to six on the third. Each seta was finely serrated along its anterior edge. The head bore four pigmented eyespots, all on the dorsal side, plus a less regular pigment spot between the median and lateral eyespot on each side. Brown pigment granules were also scattered through the anterior part of the head. The apex of the head bore three motionless cilia. Other cilia, and cirri apparently made up of fused cilia, were located on the head as shown in Figures 1 and 2. The vestibule, the pharynx or esophagus, and the entire intestine were lined with active cilia. The cilia on the third setiferous segment did not form a continuous band; there were both mid-dorsal and mid-ventral gaps. The last segment did not have setae, but bore a group of large cilia (the "grasping cilia" of Wilson, 1928) on each lateral margin, and a number of motionless cilia projecting straight back from the posterior end of the body. The lateral cilia were not seen to hold setae in the manner described by Wilson. The posterior cilia apparently correspond to the two "sensory cilia" on the posterior end of *P. ciliata* larvae as

FIGURE 1. Newly-emerged planktonic larva of *Polydora websteri* Hartman, dorsal view, alive. Camera lucida drawing under 430x magnification. Actual length 0.38 mm.

FIGURE 2. Newly-emerged planktonic larva of *Polydora websteri* Hartman, ventral view, alive. Camera lucida drawing under 430x magnification. Actual length 0.35 mm. Setae omitted.

FIGURE 3. Planktonic larva of *Polydora websteri* two days after emergence from the parent's tube, ventral view, alive. Camera lucida drawing under 430x magnification. Actual length 0.40 mm. Setae omitted. Cilia shown on third setiferous segment are actually on dorsal side.

FIGURE 4. Horseshoe-shaped burrows of young *Polydora websteri* under scales on outside surface of shell of a two-months-old oyster from Bassa Bassa Bay, Louisiana. Scale line = 1.0 mm. Camera lucida drawing.

FIGURE 5. Horseshoe-shaped burrow of young *Polydora websteri* opening on margin of upper valve of shell of two-months-old Bassa Bassa Bay oyster; it is seen on the inside surface of the shell, where a small "mud blister" is beginning to form. Scale as in Figure 6. Camera lucida drawing.

FIGURE 6. Straight and slightly curved grooves excavated by young *Polydora websteri* on flat outside surface of a two-months-old oyster from Bassa Bassa Bay. The margin of the flat valve is at top of this camera lucida drawing. Scale line = 1.0 mm.

described by Wilson (1928). In the third setiferous segment, brown pigment granules (black *en masse)* formed sometimes one continuous band and sometimes two transversely elongated lateral spots. Pigment granules around the rectum, in the extreme posterior end of the worm, seemed to form sometimes one and sometimes two spots.

On November 19, two days after emergence from the parental tubes, the *P. websteri* larvae had reached the stage shown in Figure 3. By this time some of the provisional setae had been shed, and there was a further development of large cirri, as shown.

Newly-emerged larvae, stained while alive with very dilute neutral red, showed a number of dorsal gland cells in a regular pattern. The gland cells stained red in contrast to the general pinkish or very light reddish brown of other tissues, and the fluid contents of the intestine became yellow in neutral red stain. Numerous phytoflagellates and diatoms were seen in the intestine. The flagellates, at least, were in various stages of digestion.

The embryos of *P. websteri* develop in egg sacs within the tubes made by the parent worms. The larvae, at least in early stages, are similar to the larvae of *P. ciliata* as described by Wilson (1928). They emerge from the tubes in the same stage, with three seta-bearing segments, while some other species *(P. hoplura, P. natrix,* etc.) remain in the egg sacs until they have developed to a much later stage, with tentacles and fifteen or more segments. However, the ciliation of the head in *P. websteri* is unlike that in *P. ciliata* as shown in Wilson's figures, and the number of provisional setae on each setiferous segment is different. Wilson (1928) saw eighteen setae in the first bristle bundle, about nine in the second, and about six in the third. Wilson says that "there is a sensitive cilium on each side of the anus" and shows only two cilia on the posterior end in all stages of the larval *P. ciliata,* while the larval *P. websteri* has numerous cilia surrounding the anus. There seems to be much less pigment around the rectum in *P. ciliata* than there is in *P. websteri.* The larva of *P. hoplura* Claparède in the stage with three seta-bearing segments, as described and pictured by Wilson (1928), has conspicuously different pigmentation: there is a prominent spot of black pigment on each side between the first and second bristle bundles, and a pigment spot posterior to the spot in the third setiferous segment, in addition to the pigment around the anus. The larvae of *P. hoplura* are not normally released from the parental tubes until they have tentacles and at least seventeen setiferous segments, according to Wilson.

Hannerz (1956) has recently reviewed the larval development of the European species of *Polydora* and has described some larvae not previously known. However, his pictures and descriptions all concern more advanced stages than those described here. Hartman (1941) pictured the larva of *Polydora citrona* Hancock in the stage with three setiferous segments. This was the oldest stage found in the tubes inhabited by the adults; it was stated to have no pigment spots other than the four eyespots. Hartman (1941) also pictured a planktonic larva in the stage with three setiferous segments, which she believed to be the larva of *Polydora ligni* Webster. Hartman's larva differs from the larva of *P. websteri* in having another pair of transversely-placed pigment spots posterior to the pigment spots that lie between the levels of the second and third bundles of setae, as well as in other less conspicuous details. Later stages, believed by Hartman to belong to *P. ligni,* were assigned by Hannerz to *P. ciliata,* on the basis of their possession of "seven pairs of band-shaped dorsal melanophores" (Hannerz, 1956, p. 110).

Hartman (1951) listed six species of *Polydora* known to occur in the Gulf of Mexico. Numerous specimens extracted from burrows in Louisiana oyster shells were sent to Dr. Olga Hartman in 1947, and all were identified as *P. websteri.* During the next few years an intensive study of *Polydora* as a pest of oysters was conducted in Louisiana waters by J. G. Mackin and associates (Mackin and Cauthron, 1952), and thousands of "mudworms" were extracted from oyster shells and examined. No species other than *P. websteri* was recognized until May, 1949, when Dan A. Wray found a few individuals in shells of oysters in Bayou Rigaud. Grand Isle, which differed from *P. websteri* by having large hooks in posterior segments and no conspicuous cup-like structure on the posterior end. These were identified as *Polydora hamata* Webster, 1879, by Dr. Hartman. Bayou Rigaud is in the extreme lower end of Barataria Bay, close to the deep pass into the Gulf of Mexico. *Polydora hamata* was never found farther up the bay. Bay Chene Fleur lies in the brackish-water tidal marsh to the east of the upper end of Barataria Bay, and is characterized by having long periods of low salinity, near the lower limit of the oyster's range of tolerance. Many of the worms in the Bay Chene Fleur oyster which was the source of the larvae studied November 17-19, 1947, were examined microscopically by the author and identified as *P. websteri,* and no other species was found, so it seems virtually certain that the larvae described here are the larvae of *P. websteri.* It may be worth

mentioning also that *Polydora hamata* is very similar to *P. hoplura,* so similar that Carazzi (1893) considered *P. hamata* a synonym. One would expect, therefore, that the larvae of *P. hamata* probably remain within the egg sacs until they have tentacles and seventeen or more segments, like the larvae of the closely related European species.

The Planktonic Period and Settling of the Larvae

As noted, *P. websteri* larvae with three setiferous segments were seen emerging from the tubes of the parent worms at a water temperature of 24°C. The water temperatures in Barataria Bay were 18 to 19°C on November 19, 1947, but they had been over 24°C during the first week of November. In early January, 1948, many *P. websteri* of larval or near-larval size, the smallest with seventeen segments, were found in Barataria Bay oyster shells by Dr. Mackin and the writer. Water temperatures in the bay then ranged between 12 and 18°C. At the same time, the writer found egg cases containing numerous embryos with approximately eight cells in the tubes of older Polydoras. At the end of January mudworms of seventeen segments were still invading oyster shells, and worms with seventeen segments were seen swimming by Dr. Mackin. Bay water temperatures were 7 to 10°C.

On February 28, 1948, the writer caught *Polydora* larvae of five to twelve segments in a plankton net at Bay Sainte Elaine, Terrebonne Parish, Louisiana; the water temperature was 21°C. On May 6 and May 8, 1948, the writer found many *Polydora* larvae of five to seventeen segments in plankton at Bay Sainte Elaine and at Grand Isle, at water temperatures of 25 to 27°C. The larger planktonic worms had three "boring spines" on the fifth setiferous segment, and hooklets beginning on the seventh segment.

On July 16, 1948, Dr. Mackin reported that larval or post-larval mudworms in great numbers were attacking oysters, but that most of the worms were building mud tubes on the outside surfaces of the shells: only a few were found to be excavating burrows in the margins of the shells. The writer studied the *Polydora* infestations of Bay Chene Fleur and Bassa Bassa Bay oysters July 22-25, 1948, and found that about half the population consisted of very young worms, some with as few as seventeen segments. The water temperature in the last half of July was 28 to 31°C. Oysters placed in trays in lower Barataria Bay on August 19, 1948, after being dewormed with phenol, became reinfested by numerous mudworms before September 23, 1948, when

they were examined. The water temperatures ranged from 25 to 30°C during this period.

It will be seen from these notes that planktonic *Polydora* larvae may be found in Louisiana waters in any season of the year, at water temperatures ranging from approximately 10 to more than 30°C. Although the larger *Polydora* larvae seen in 1948 were not identified to species, it seems probable that most of them belonged to *P. websteri,* which was many times more abundant than *P. hamata.* Hannerz (1956) found the planktonic stages of each species of *Polydora* to have a characteristic seasonal occurrence; for instance *P. coeca* larvae occurred in March and April only, while *P. ligni* larvae were found only from late July to the end of October. However, Hannerz worked in Sweden, and Louisiana conditions are very different. To the writer it seems probable that *P. websteri* larvae develop within the egg cases throughout the year, rapidly in warm weather, and slowly or not at all during the relatively short periods of low temperature, and that larvae emerge from the parental tubes whenever water temperature approaches 25°C. In shallow Louisiana bays temperatures up to 25°C may occur, at least for short periods, in every month except December, January, and February; 20°C may occur in any month of the year. Once emerged, planktonic larvae probably continue to swim until they reach the settling stage, developing faster at high temperatures and slower at low temperatures, so that cold weather has the effect of extending the length of the planktonic period. Since the largest mudworms found in plankton and the smallest on oyster shells have seventeen segments, it seems virtually certain that *P. websteri* settles on oyster shells and becomes a tube dweller at approximately the seventeen-segment stage. Settling may occur at any time of the year, but Dr. Mackin found the heaviest new infestations of oysters in warm weather.

Figures 4, 5, and 6 show excavations made by very young *Polydora websteri* on two-months-old oyster spat. The young oysters were taken from Bassa Bassa Bay on July 23, 1948, and scrubbed clean with a brush. Figure 4 shows horseshoe-shaped burrows under the scales on the rough outside surface of a young oyster. Figure 5 shows a horseshoe-shaped burrow on the smooth inside surface of the upper valve of a young oyster, with the two openings of the burrow close together on the extreme margin of the valve. This is the beginning of a "mud blister" which will become much larger as the worm grows and enlarges its burrow. Mud is packed around and between the two branches of the horseshoe-shaped burrows shown in Figures 4 and 5. Figure 6

shows grooves cut into the outside surface of a young oyster's shell where there are no scales; nearly straight mud tubes may be built on such a surface, but they become U-shaped as the worm grows and the "groove" is deepened into a pit. The worms and the outside extensions of their mud tubes had been scrubbed off in order to make these drawings. Although the actual process was not observed, the evidence indicates that *P. websteri* settles on shells, starts its excavations, and builds its mud tubes in the manner described for *P. hoplura* by Wilson (1928) and for *P. ciliata* by Korringa (1951, 1951a) and Hannerz (1956).

Conclusions

1. The larvae of *Polydora websteri* Hartman develop in chains of egg cases within the mud-lined burrows of the parent worms.
2. Larvae leave the parental burrows, swimming rapidly, when they reach the stage with three seta-bearing segments, provided that the water temperature is approximately 24°C or higher.
3. *P. websteri* larvae in the three-segmented stage are positively phototaxic.
4. The stage of *P. websteri* with three setiferous segments differs from the corresponding stage of *P. ciliata* in number and location of cilia, in number of setae, and in pigmentation.
5. Planktonic larvae of *Polydora,* presumably *P. websteri,* are found in Louisiana waters at all seasons, at water temperatures ranging from near 10 to over 30°C. The largest planktonic larvae have seventeen segments.
6. Larval or early post-larval *P. websteri* settle on oyster shells, and begin excavation of burrows and construction of mud tubes at all seasons, but especially in warm weather. The smallest worms on oysters have seventeen segments.
7. The burrows which *P. websteri* excavates in oyster shells begin as shallow grooves on the outside surface or on the margin of the shell. As the worm grows, the groove is deepened into a pit in which the worm lies in a horseshoe shape. The space between the wings of the horseshoe, and around the tube in which the worm moves, is packed with mucus-cemented mud or detritus. A mucus-cemented tube of mud projects from each end of the horseshoe-shaped tube, on the outside surface of the oyster shell.

LITERATURE CITED

Carazzi, D.
1893. Revisione del genere Polydora Bosc e cenni su due specie che vivono sulle ostriche. Mitt. Zool. Stat. Neapel, *11* (1-2): 4-45.

Hannerz, L.
1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil, and Poecilochaetidae n. fam. in the Gullmar Fjord (Sweden). Zool. Bidrag Uppsala, *31*: 1-204.

Hartman, Olga
1941. Some contributions to the biology and life history of Spionidae from California. Allen Hancock Pacific Exped. 1932-40, *7* (4): 289-321.
1951. The littoral marine annelids of the Gulf of Mexico. Publ. Inst. Mar. Sci., *2* (1): 7-124.

Korringa, P.
1951. Polydora als vijand van de oestercultuur. Visserij-Nieuws, Suppl. 2, Jaarg. 3, No. 10: 1-12. (English translation by S. H. Hopkins, corrected by Korringa, mimeo.)
1951a. The shell of *Ostrea edulis* as a habitat. Arch. Neerland. Zool., *10* (1): 31-152.

Leloup, E.
1937. Contributions a l'étude de la faune belge. VIII. Les dégâts causés par le ver polychète *Polydora ciliata* (Johnston) dans les coquilles des bigorneaux et des huitres. Bull. Mus. Hist. nat. Belg. *13* (33): 1-4.

Leschke, M.
1903. Beitraege zur Kenntnis der pelagischen Polychaeten Larven der Kieler Foehrde. Wiss. Meeresunters. Abt. Kiel & Leipzig, *7*: 113-114.

Loosanoff, V. L. and J. B. Engle
1943. Polydora in oysters suspended in the water. Biol. Bull., *85*: 69-78.

Lunz, G. R., Jr.
1940. The annelid worm, Polydora, as an oyster pest. Science, *92*: 310.
1941. Polydora, a pest in South Carolina oysters. J. Elisha Mitchell Sci. Soc., *57*: 273-283.

Mackin, J. G. and Fred F. Cauthron
1952. Effect of heavy infestations of *Polydora websteri* Hartman on *Crassostrea virginica* (Gmelin) in Louisiana. Natl. Shellf. Assoc. Convention Addr. *1952*: 14-24.

Nelson, T. C. and L. A. Stauber
1940. Observations of some common polychaetes on New Jersey oyster beds with special reference to *Polydora*. (Abstract), Anat. Rec. *78*: 102.

Owen, H. M.
1957. Etiological studies on oyster mortality.
II. *Polydora websteri* Hartman—(Polychaeta: Spionidae). Bull. Mar. Sci. Gulf & Carib., *7* (1): 35-46.

Soederstrom, A.
1920. Studien ueber die Polychaetenfamilie Spionidae. Inaugural Dissertation, Uppsala. 286 pp.

Whitelegge, Thomas
1890. Report on the worm disease affecting oysters on the coast of New South Wales. Rec. Austr. Mus., *2:* 41-54.

Wilson, D. P.
1928. The larvae of *Polydora ciliata* Johnston and *Polydora hoplura* Clapa-Rède. J. Mar. Biol. Assoc. U. K., *15:* 567-603.

UNDERWATER SOUNDS PRODUCED BY TWO SPECIES OF TOADFISH, *OPSANUS TAU* AND *OPSANUS BETA*[1]

WILLIAM N. TAVOLGA
Department of Animal Behavior, The American Museum of Natural History, New York

Abstract

A comparison of the horn-like or "boat-whistle" sounds produced by two species of toadfish, reveals that there are differences in pitch, harmonic content, duration and repetition rate. The sounds were recorded in the field and analyzed with a sound spectrograph.

Introduction

The "boat-whistle" sound of the common toadfish, *Opsanus tau* (Linnaeus), has been described by Fish (1954). A related species from the Florida Gulf coast, *Opsanus beta* (Goode and Bean), locally known as the dog-fish or dog-toad, also utters a similar sound. Both species produce a variety of grunts and growls when disturbed, but the horn-like sounds are often associated with the breeding season. This report presents analyses and comparisons of these sounds as produced by both species.

The author is indebted to Dr. Eugenie Clark, director of the Cape Haze Marine Laboratory, for making the facilities of the laboratory available. Mr. Robert Laupheimer, of the Institute of Mathematical Sciences, and Mr. Nathaniel Tillman, of the Electrical Engineering Department of the City College, were extremely helpful in the instrumentation involved in this project. Dr. Louis Gerstman, of the Haskins Laboratories, gave many valuable suggestions on the analysis of the sounds. The author is also grateful to Dr. Lester R. Aronson, of The American Museum of Natural History, for his comments on the manuscript.

Equipment

The hydrophone that was used to pick up the sounds was a Barium Titanate transducer, Model LF-400, manufactured by the Chesapeake Instrument Corporation, Shadyside, Maryland. A low-noise voltage amplifier was designed by Mr. Robert Laupheimer for this transducer.

[1]These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and The American Museum of Natural History. Contract No. NR 163-322.

The analysis of the sounds was made from tape recordings with a sound spectrograph (Sonagraph, Kay Electric Company), and a cathode ray oscilloscope. Details of the equipment are reported in another paper (Tavolga, 1958).

Sounds of Opsanus Tau

"Boat-whistle" sounds were heard in late August, and recorded at Matanzas Inlet, 15 miles south of St. Augustine, Florida. At least five different sources could be detected within the range of the equipment. No specimens were collected at this time. *O. tau* is known to occur in this region, and the author has collected numerous specimens on previous occasions. The "boat-whistle" call of this species is well-known and distinctive. It is even included on a commercially available disc recording. This is the only species of toadfish in this area (Walters and Robins, M.S.).

The hoot-like sounds of the breeding season are always accompanied by short grunts. Each hoot is usually preceded by a grunt. A sound spectrogram of one such sound is shown in Fig. 1. The initial grunts are non-harmonic and they often consist of a double pulse of sound. About 90 per cent of the sound energy is in the 240 to 300 cps. range. No distinct harmonics could be detected either in the spectrograms or oscilloscope observations. The duration of each sound pulse comprising a grunt varies from 50 to 70 milliseconds.

The predominant frequency in the hoot portion of these sounds is 250 cps., and about 75 per cent of the sound energy is at this frequency. Two very weak harmonics can be detected, one at 500 cps. and the other at 750 cps. The harmonics are discontinuous through the duration of the hoot. The length of an average hoot—excluding the initial grunt—is 650 milliseconds, with a range of 600 to 700 milliseconds.

Sounds of Opsanus Beta

The recordings described below were made in late August, 1957, at Gasparilla Sound, a few miles south of Lemon Bay, Englewood, Florida. At most four individuals could be heard, and the hoots occurred sporadically both during the day and at night. One individual sounded close to the laboratory dock where the hydrophone was placed and the others were very faint and distant. After some trials, a single specimen was captured on hook and line. It proved to be a male of *Opsanus beta* of exceptionally large size. The standard length of this specimen

was 281 mm, whereas 130 mm is considered average (Breder, 1941). For several days after the capture of the above animal, the only hoots that could be detected were faint and distant, then the loud close-range sounds appeared again. It is possible that a new animal had now moved into the vacated area. The only other toadfish of this area is *O. pardus* (Goode and Bean), and this is reported to be a deeper water form (Walters and Robins, M.S.). No *pardus* have been collected in the Gasparilla Bay area.

The hoots of *O. beta* are distinctly different from those of *O. tau* in being shorter, higher pitched and frequently double. The initial grunts are also non-harmonic, but they show a wider distribution of frequencies—from 100 to 1800 cps. Sound spectrograms (Fig. 2) show the maximum sound energy to be in the 100 to 500 cps. range. The duration of each grunt ranges from 30 to 70 milliseconds, with a mean of 55 milliseconds.

The hoot portion of the sound is clearly harmonic. The fundamental frequency is at 350 cps. The next three harmonics are distinct, the fourth is often absent, and the fifth is weak. The following table shows

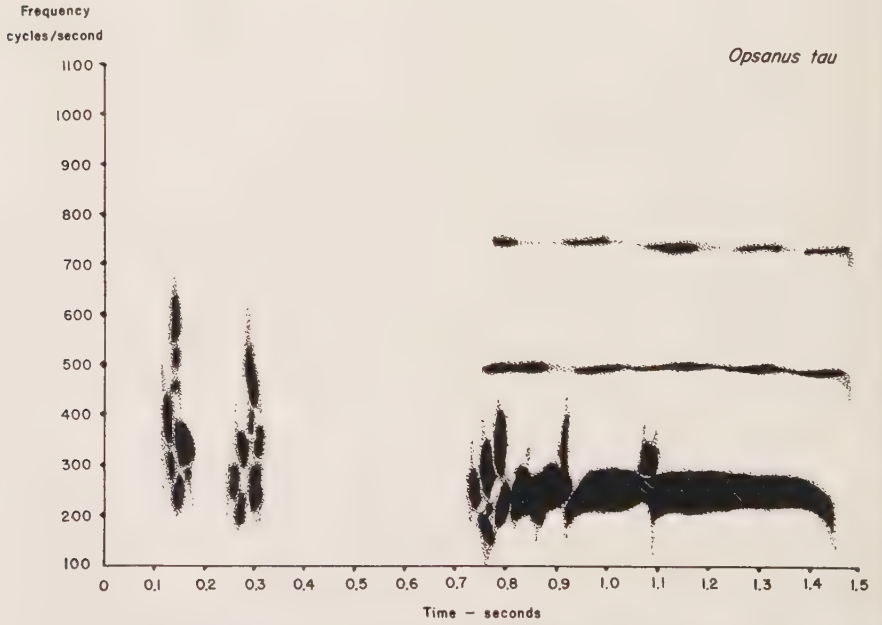


FIGURE 1. Drawing of sound spectrogram representing a double grunt followed by a long hoot produced by *Opsanus tau*, recorded in Florida.

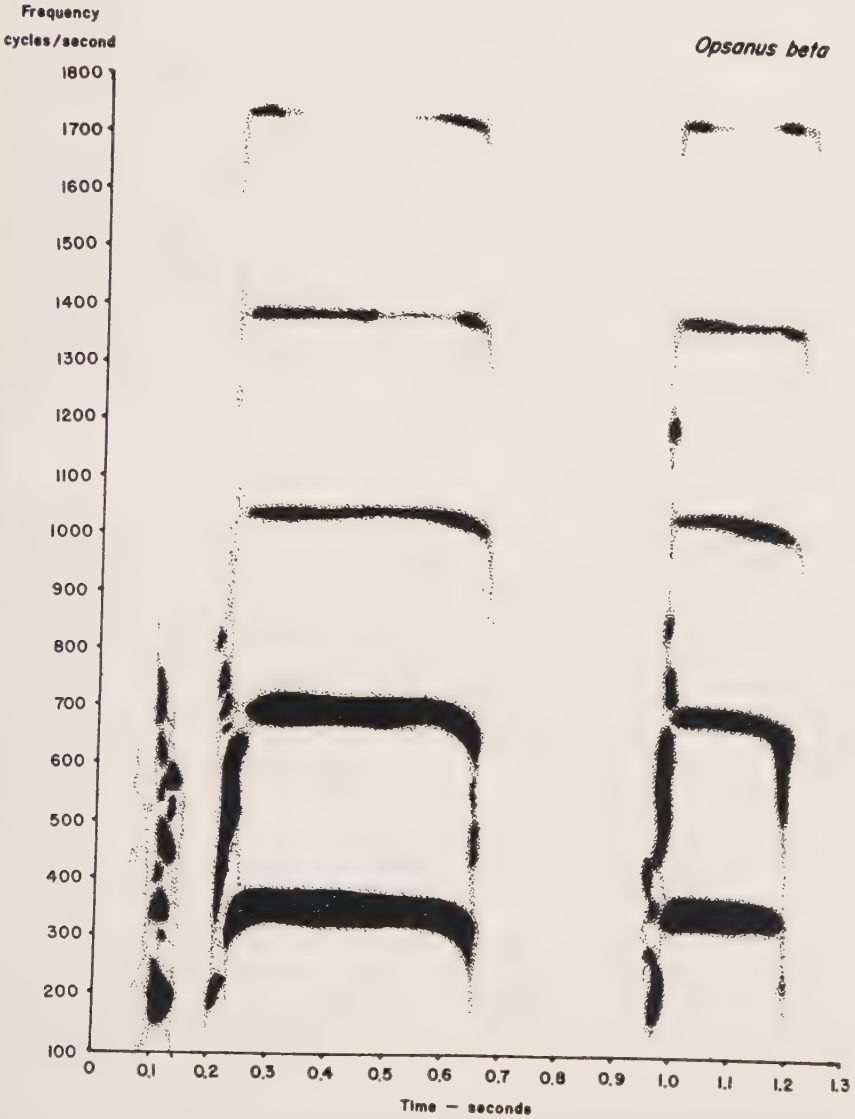


FIGURE 2. Drawing of sound spectrogram representing a grunt followed by two hoots produced by *Opsanus beta*, recorded in Florida. The harmonic at 2100 cps. is not shown here.

the component frequencies and the percentages of total sound energy of each harmonic:

Frequency	*Per Cent Sound Energy*
350 cps.	25%
700	24
1050	17
1400	20
1750	1 (or absent)
2100	13

The duration of the hoots ranges from 350 to 410 milliseconds (average—370 ms.), for the single blasts and the first of the double blasts. The secondary hoots, when present, are shorter, ranging from 160 to 200 ms. (average—170 ms.).

Discussion

The mechanism by means of which the toadfish produces sounds has been described by Tower (1908) and Fish (1954), for *Opsanus tau*. The air bladder is bilobed and possesses a considerable intrinsic musculature. Electrical stimulation elicits only the non-harmonic grunts. Thus far the boat-whistle sounds have been produced by captive animals on only one occasion (Fish, 1954).

Fish's (1954) description of the hoots of *O. tau* is similar to the present one in that the hoot is almost a pure tone with two very weak harmonics at octave intervals. The fundamental frequency for animals from the vicinity of Kingston, Rhode Island, was 325 cps., whereas the *O. tau* recorded from Florida produced a distinctly lower pitch at 250 cps. Whether this difference can be ascribed to geographical variation, size differences, seasonal differences, or some combination of these is unknown. In their taxonomic review of the toadfishes, Schultz and Reid (1937) redescribed *Opsanus beta* as the Florida Gulf coast species. They indicated a considerable amount of geographical variation for the Atlantic coast *O. tau* and also described the occurrence of intermediate specimens from around the southern end of the Florida peninsula.

The exact relationship of the sounds to spawning behavior is not known. The two species have similar breeding habits, as described by Gudger (1910) for *O. tau*, and Breder (1941) for *O. beta*. The animals are solitary and territorial, and the adherent eggs are deposited

on the surface of some object used as a nest. The spawning temperature is between 60° and 70° F. Thus the spawning season varies from the winter months in Florida to the summer months in New York and northward (Breder, 1941). It is probable that the sounds described here would not be representative of the spawning season, since they were recorded in August. It should be noted, however, that no boat-whistle sounds were detected through the months of June and July, 1957, during the author's stay in Florida. The first of the *O. beta* hoots was heard on August 20th. It is possible that the sound production may precede the actual breeding season.

It is not known whether both sexes produce the hoots or not. Fish (1954) believes that the ability to produce sounds is alike in both males and females. Both sexes are definitely known to produce the grunts and growls, and there are no apparent structural differences in the swim bladders of the two.

Summary

Horn-like or "boat-whistle" sounds of the toadfish, *Opsanus tau,* were recorded from the Atlantic coast of Florida. Each sound consists of a short grunt followed by a hoot. The grunt is non-harmonic and contains frequencies from 240 to 300 cps. The hoot is almost a pure tone of 250 cps. and 600 to 700 milliseconds in duration.

Similar "boat-whistle" sounds are produced by *Opsanus beta,* a Florida Gulf coast species. The hoots are shorter, higher pitched, and frequently double. Harmonic analysis shows the presence of five component frequencies at 350, 700, 1,050, 1, 400 and 2,100 cps. The harmonic at 1,750 cps. is usually weak or absent. The duration of each hoot averages 370 milliseconds (170 milliseconds for the secondary hoots, when present). Short, non-harmonic grunts precede the hoots.

LITERATURE CITED

Breder, Charles M., Jr.
1941. On the reproduction of *Opsanus beta* Goode & Bean. Zoologica, N. Y., *26* (3): 229-232.

Fish, Marie Poland
1954. The character and significance of sound production among fishes of the western North Atlantic. Bull. Bing. oceanogr. Coll., *14* (3): 1-109.

Gudger, E. W.
1910. Habits and life history of the toadfish (*Opsanus tau*). Bull. U. S. Bur. Fish., *28*: 1071-1109.

Schultz, Leonard P. and E. D. Reid
1937. The American Atlantic toadfishes of the genus *Opsanus*. Copeia, 1937 (4): 211-212.

Tavolga, William N.
1958. The significance of underwater sounds produced by males of the gobiid fish, *Bathygobius soporator*. Physiol. Zool., in press.

Tower, R. W.
1908. The production of sound in the drumfishes, the sea robin and the toadfish. Ann. N. Y. Acad. Sci., *18* (5): 149-180.

Regional Bibliography for 1957

The scope of the bibliography is limited in general to the area which includes the southeastern United States, the Gulf of Mexico, and the Caribbean, but papers of more general interest may be listed in cases where they have some special significance to the area.

The degree of comprehensiveness of the bibliography is to a large extent dependent upon the cooperation of workers in the Marine Sciences of this area. *It is therefore hoped that individuals and organizations will aid by sending copies of all relevant publications to the Editor.* Any omissions brought to the attention of the Editor will be included in subsequent issues.

It has been found convenient to use a modification of the classification used by the Journal du Conseil Bibliography for present purposes.

The Editorial staff wishes to acknowledge the valuable assistance given by a number of investigators in compiling the bibliography.

General

Anonymous
1957. Regional bibliography for 1956. Bull. Mar. Sci. Gulf & Carib., *7*(3): 276-287.

Breuer, Joseph P.
1957. An ecological survey of Baffin and Alazan Bays, Texas. Publ. Inst. Mar. Sci., *4*(2): 134-155.

Simmons, Ernest G.
1957. An ecological survey of the upper Laguna Madre of Texas. Publ. Inst. Mar. Sci. *4*(2): 156-200.

Hydrography

Hela, Ilmo, Clarence A. Carpenter, Jr. and J. Kneeland McNulty
1957. Hydrography of a positive, shallow, tidal bar-built estuary (Report on the hydrography of the polluted area of Biscayne Bay). Bull. Mar. Sci. Gulf & Carib., *7*(1): 47-99.

Reid, George K.
1957. Biologic and hydrographic adjustment in a disturbed Gulf coast estuary. Limnol. and Oceanogr., *2*(3): 198-212.

Physical Properties

General

Arons, A. B. and Henry Stommel
1956. A Beta-plane analysis of free periods of the second class in meridional and zonal oceans. Deep-Sea Res., *4*(1): 23-31.

Bein, Selwyn Jack
1957. The relationship of total phosphorus concentration in sea water to Red Tide blooms. Bull. Mar. Sci. Gulf & Carib., *7*(4): 316-329.

KINSMAN, B.
1957. Proper and improper use of statistics in Geophysics. Tellus, 3: 408-418.
ODUM, HOWARD T.
1957. Strontium in natural waters. Publ. Inst. Mar. Sci., *4*(2): 22-37.
1957. Biogeochemical deposition of strontium. Publ. Inst. Mar. Sci., *4*(2): 38-114.
THOMAS, W. H., E. F. CORCORAN AND E. G. SIMMONS
1957. Organic productivity of Mississippi delta waters. Inst. Mar. Res., U. of Calif., Ref. 57-3.
TUREKIAN, KARL K.
1957. Salinty variations in sea water in the vicinity of Bimini, Bahamas, British West Indies. Amer. Mus. Novitates No. 1822: 1-12.
VON ARX, W. S.
1957. An experimental approach to problems in physical oceanography. *In* Progress in Physics and Chemistry of the earth, Vol. 2. Pergamon Press, New York.

Currents and Oceanic Circulation

HULBURT, EDWARD M.
1957. Sea-level fluctuations at Charleston, South Carolina. Bull. Mar. Sci. Gulf & Carib., *7*(1): 21-34.
MOORE, FRANK W., JR., CHARLES L. BRETSCHNEIDER AND DONALD W. HOOD
1957. A method for measuring eddy diffusion in coastal embayments. Publ. Inst. Mar. Sci., *4*(2): 14-21.
MOSBY, HAKON
1955. Note on wire-angle in oceanography. J. Mar. Res., *14*(3): 259-260.
STOMMEL, HENRY
1957. Florida Straits transports, 1952-1956. Bull. Mar. Sci. Gulf & Carib., *7*(3): 252-254.
STOMMEL, H.
1957. A survey of ocean current theory. Deep-Sea Res., *4*(3): 149-184.

Geography, Geology and Bottom Deposits

CURL, HERBERT, JR.
1957. Changes in bottom topography off Alligator Harbor since 1889. Quart. J. Fla. Acad. Sci., *20*(3): 205-208.
EMILIANI, CESARE
1957. Temperature and age analysis of deep-sea cores. Science, *125*(3244): 383-387.
FRASSETTO, ROBERT AND JOHN NORTHROP
1957. Virgin Islands bathymetric survey. Deep-Sea Res., *4*(2): 138-146.
KORNICKER, LOUIS S. AND EDWARD G. PURDY
1957. A Bahamian faecal-pellet sediment. J. Sediment. Petrol., *27*(2): 126-128.
LOWENSTAM, HEINZ A.
1955. Aragonite needles secreted by algae and some sedimentary implications. J. Sediment. Petrol., *25*(4): 270-272.
LUND, E. J.
1957. Self-silting by the oyster and its significance for sedimentary geology. Publ. Inst. Mar. Sci., *4*(2): 320-327.
SHEPARD, FRANCIS P. AND GENE A. RUSUACH
1957. Texas Bay sediments. Publ. Inst. Mar. Sci., *4*(2): 5-13.

Plankton

Zooplankton

Dales, R. Phillips
1956. An annotated list of the pelagic Polychaeta. Ann. Mag. Nat. Hist., *9*(100): 289-304.
Fleminger, Abraham
1957. New calanoid copepods of *Pantella* Dana and *Labidocera* Lubbock with notes on the distribution of the genera in the Gulf of Mexico. Tulane Stud. Zool., *5*(2): 17-34.
Fleminger, Abraham and Thomas E. Bowman
1956. A new species of *Candacia* (Copepoda: Calanoida) from the Western North Atlantic ocean. Proc. U. S. Nat. Mus., *106*(3370): 331-337.
Suarez Caabro, Jose A.
1957. Investigaciones sobre el plancton marino de Cuba. Noverim, *2*(6): 1-15.

Phytoplankton

Ragotzkie, Robert A. and Laurence R. Pomeroy
1957. Life history of a dinoflagellate bloom. Limnol. & Oceanogr., *2*(2): 62-69.

Biology of Plankton

Bsharah, Lewis
1957. Plankton of the Florida Current. V. Environmental conditions, standing crop, seasonal and diurnal changes at a station forty miles east of Miami. Bull. Mar. Sci. Gulf & Carib., *7*(3): 201-251.
Moore, H. B. and D. L. O'Berry
1957. Plankton of the Florida Current. IV. Factors influencing the vertical distribution of some common copepods. Bull. Mar. Sci. Gulf & Carib., *7*(4): 297-315.

Bottom Fauna and Flora

Systematics and Development

Abbott, R. Tucker
1957. *Prunum roscidum* in New Jersey. Nautilus, *71*(2): 52-53, 1 pl.
Allen, M. Jean
1957. The breeding of polychaetous annelids near Paraguera, Puerto Rico. Biol. Bull., *113*(1): 49-57.
Bayer, Frederick M.
1957. Additional records of western Atlantic octocorals. J. Wash. Acad. Sci., *47*(11): 379-390.
Broad, A. C.
1957. Larval development of *Palaemonetes pugio* Holthuis. Biol. Bull., *112*(2): 144-170.
Costlow, John D. and C. G. Bookhout
1957. Larval development of *Balanus eburneus* in the laboratory. Biol. Bull., *112*(3): 313-324.
Deichmann, Elisabeth
1957. The littoral holothurians of the Bahama Islands. Amer. Mus. Novitates, No. 1821: 1-20.

Erdman, Donald S.
1957. Vagabond cuttle-bones. Nautilus, *70*(3): 106-107.
Hollister, S. C.
1957. On the status of *Fasciolaria distans* Lamarck. Nautilus, *70*(3): 73-84, pl. 6.
Menzel, R. Winston
1956. Some additional differences between *Crassostrea virginica* and *Ostrea equestris* in Gulf of Mexico waters. Proc. Nat. Shellfish Assoc., *46*(1955): 76-81.
Menzies, Robert J.
1956. New bathyal Isopoda from the Caribbean with observations on their nutrition. Breviora Mus. Comp. Zool., 63: 1-10.
1957. The marine borer family Limnoriidae (Crustacea: Isopoda). Part I. Northern and Central America: Systematics, distribution and ecology. Part II. Additions to the systematics. Bull. Mar. Sci. Gulf & Carib., *7*(2): 101-200.
Moore, Donald R.
1957. A note on *Cuna dalli*. Nautilus, *70*(4): 123-125, pl. 8.
Nowell-Usticke, G. W.
1957. Marine shells from Altona Lagoon, St. Croix, Virgin Islands. Nautilus, *70*(4): 113-116.
Robertson, Robert
1957. A study of *Cantharus multangulus* (Philippi), with notes on *Cantharus* and *Pseudoneptunea* (Gastropoda: Buccinidae). Notulae Naturae, No. 300: 1-10.
1957. The subgenus *Halosephus* Rehder, with notes on the Western Atlantic species of *Turbo* and the subfamily Bothropomatinae Thiele. J. Wash. Acad. Sci., *47*(9): 316-319.
Squires, Donald F.
1957. New species of caryophyllid corals from the Gulf coast Tertiary. J. Paleontol., *31*(5): 992-996.
Voss, Gilbert L.
1957. Observations on *Ornithoteuthis antillarum* Adam, 1957 an ommastrephid squid from the West Indies. Bull. Mar. Sci. Gulf & Carib., *7*(4): 370-378.
Warmke, Germaine L.
1957. List of the Conidae of Puerto Rico. Nautilus, *71*(1): 1-3.
Woodring, W. P.
1957. *Muracypraea*, a new subgenus of *Cypraea*. Nautilus, *70*(3): 88-90.

Distribution and Ecology

Anisgard, Harry W.
1956. *Eorupertia* in the Eocene of Venezuela. Contrib. Cushman Fdn., *7*(2): 48-59.
1957. *Eorupertia bermudezi*, a new foraminifer from the middle Eocene of Cuba. Contrib. Cushman Fdn., *8*(1): 1-8.
Bronnimann, Paul
1956. Upper Cretaceous orbitoidal Foraminifera from Cuba. Conrtib. Cushman Fdn., *7*(2): 60-66.
Chin, Edward and Donald M. Allen
1957. Toxicity of an insecticide to two species of shrimp, *Penaeus aztecus* and *Penaeus setiferus*. Texas J. Sci., *9*(3): 270-278.

Crane, Jocelyn
1957. Basic patterns of display in fiddler crabs (Ocypodidae: Genus *Uca*). Zoologica, N. Y., *42*(2): 69-82, 1 pl.
Darnell, R. M.
1956. A note on the occurrence of the pink shrimp, *Penaeus duorarum,* in Louisiana waters. Ecology, *37*(4): 844-846.
Dawson, C. E.
1957. *Balanus* fouling of shrimp. Science, *126*(3282): 1068.
Gunter, Gordon and R. Winston Menzel
1957. The crown conch, *Melongena corona,* as a predator upon the Virginia oyster. Nautilus, *70*(3): 84-87.
Krau, Luisa
1956. A existencia de *Clypeaster latissimus* (Lamarck) no Brasil e considerações sobre *Clypeaster subdepressus* (Gray) (Clypeastroida: Echinoidea). Mem. Inst. Oswaldo Cruz, *54*(2): 413-427.
Loesch, Harold C.
1957. Studies of the ecology of two species of *Donax* on Mustang Island, Texas. Publ. Inst. Mar. Sci., *4*(2): 201-227.
Lunz, G. Robert
1957. Notes on rock shrimp, *Sicyonia brevirostris* (Stimpson) from exploratory trawling off the South Carolina coast. Contrib. Bears Bluff Lab., 25: 1-10.
McGinty, Paul L. and Thomas L.
1957. Dredging for deep-water shells in southern Florida. Nautilus, *71*(2): 37-47.
Phleger, Fred B. and Robert R. Lankford
1957. Seasonal occurrences of living benthonic Foraminifera in some Texas bays. Contrib. Cushman Fdn., *8*(3): 93-105.
Sachs, K. N.
1957. Restudy of some Cuban larger Foraminifera. Contrib. Cushman Fdn., *8*(3): 106-120.
Sutcliffe, W. H., Jr.
1957. Observations on the growth rate of the immature Bermuda spiny lobster, *Panulirus argus*. Ecology, *38*(3): 526-529.
Turner, Henry J., Jr. and David L. Belding
1957. The tidal migrations of *Donax variabilis* Say. Limnol. & Oceanogr., *2*(2): 120-124.
Voss, Gilbert L.
1957. Observations on abnormal growth of the arms and tentacles in the squid genus *Rossia*. Quart. J. Fla. Acad. Sci., *20*(2): 129-132.
Voss, Gilbert L. and Craig Phillips
1957. A first record of *Octopus macropus* Risso from the United States with notes on its behavior, color, feeding and gonads. Quart. J. Fla. Acad. Sci., *20*(4): 223-232.

Physiology and Behavior

Costlow, John D., Jr. and E. G. Bookhout
1957. Body growth versus shell growth of *Balanus improvisus*. Biol. Bull., *113*(2): 224-232.
Demeusy, Noelle
1957. Respiratory metabolism of a fiddler crab *Uca pugilator* from two different latitudinal populations. Biol. Bull., *113*(2): 245-253.

Fingerman, Milton
1957. Relation between position of burrows and tidal rhythm of *Uca*. Biol. Bull., *112*(1): 7-20.
Fingerman, Milton, Laurence D. Fairbanks and Warren C. Plauche
1957. Body fluids of the oyster *Crassostrea virginica*. Publ. Inst. Mar. Sci., *4*(2): 284-292.
Gray, I. E.
1957. A comparative study of the gill area of crabs. Biol. Bull., *112*(1): 34-42.
Lund, E. J.
1957. A quantitative study of clearance of a turbid medium and feeding by oyster. Publ. Inst. Mar. Sci., *4*(2): 296-312.
1957. Self-silting, survival of the oyster as a closed system, and reducing tendencies of the environment of the oyster. Publ. Inst. Mar. Sci., *4*(2): 313-319.
1957. Effect of the bleedwater, "soluble fraction" and crude oil on the oyster. Publ. Inst. Mar. Sci., *4*(2): 328-341.
Lund, E. J. and Ernest Powell
1957. Note on the reflex inhibitor of water propulsion by the oyster. Publ. Inst. Mar. Sci., *4*(2): 293-295.
Mouton, James M.
1957. Sound production in the spiny lobster, *Panulirus argus* (Latreille). Biol. Bull., *113*(2): 286-295.
Odum, Howard T. and Charles M. Hoskins
1957. Metabolism of a laboratory stream microcosm. Publ. Inst. Mar. Sci., *4*(2): 115-133.
Owen, H. Malcolm
1957. Etiological studies on oyster mortality. II. *Polydora websteri* Hartmann—(Polychaeta: Spionidae). Bull. Mar. Sci. Gulf & Carib., *7*(1): 35-46.
Phleger, Fred B.
1956. Significance of living foraminiferal populations along the central Texas coast. Contrib. Cushman Fdn., *7*(4): 106-151.
Schafer, R. D. and Charles E. Lane
1957. Some preliminary observations bearing on the nutrition of *Limnoria*. Bull. Mar. Sci. Gulf & Carib., *7*(4): 281-296.
Travis, Dorothy F.
1957. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. IV. Post-ecdysial histology and histochemical changes in the hepatopancreas and integumental tissues. Biol. Bull., *113*(2): 451-479.

Parasitic Forms

Bowman, Thomas E. and Carlos Diaz-Ungria
1957. Isopodos quimotoideos parasitos de peces de las aguas venezolanos. Mem. Soc. cienc. nat. La Salle, *17*(47): 112-124.
Bullock, Wilbur L.
1957. The acanthocephalan parasites of the fishes of the Texas coast. Publ. Inst. Mar. Sci., *4*(2): 278-283.
Hargis, William J.
1956. Monogenetic trematodes of Gulf of Mexico fishes. X. The family Microcotylidae Taschenberg, 1879. Trans. Amer. Microsc. Soc., *75*(4): 436-453.

Hargis, William J.
1957. Monogenetic trematodes of Gulf of Mexico fishes. XIII. The family Gastrocotylidae Price, 1943. Trans. Amer. Microsc. Soc., *76*: 1-12
Pettibone, Marian H.
1957. Endoparasitic polychaeteus annelids of the family Arabellidae with descriptions of new species. Biol. Bull., *113*(1): 170-187.
Sparks, Albert K.
1957. Some digenetic trematodes of marine fishes of the Bahama Islands. Bull. Mar. Sci. Gulf & Carib., *7*(3): 255-265.
Sparks, Albert K. and John G. Mackin
1957. A larval trypanorhynchid cestode from commercial shrimp. Texas J. Sci., *9*(4): 475-476.
Tarjan, A. C.
1957. An emended description of the marine nematode genus *Halenchus* Cobb, 1933 (Tylenchinae). Quart. J. Fla. Acad. Sci., *20*(2): 121-125.
Yeatman, Harry C.
1957. A redescription of two parasitic copepods from Bermuda. J. Wash. Acad. Sci., *47*(10): 346-353.

Marine Plants

Johnson, T. W., Jr. and Samuel P. Meyers
1957. Literature on halophilous and hololimnic fungi. Bull. Mar. Sci. Gulf & Carib., *7*(4): 330-359.
Humm, Harold J. and R. L. Caylon
1957. The summer marine flora of Mississippi Sound. Publ. Inst. Mar. Sci., *4*(2): 228-264.
Olson, F. C. W.
1957. A seaball from Escambia County, Florida. Quart. J. Fla. Acad. Sci., *20*(1): 93-94.
Phillips, Ronald C.
1957. Notes on gametangia in *Udotea*. Quart. J. Fla. Acad. Sci., *20*(4): 253-254.

Fish

Systematics, Morphology and Development

Anderson, William W.
1957. Early development, spawning, growth, and occurrence of the silver mullet (*Mugil curema*) along the south Atlantic coast of the United States. Fish. Bull., U. S., *57*(119): 397-414.
1957. Larval forms of the fresh-water mullet (*Agonostomus monticola*) from the open ocean off the Bahamas and south Atlantic coast of the United States. Fish. Bull., U. S., *57*(120): 415-425.
Anderson, William W. and Jack W. Gehringer
1957. Physical oceanographic, biological, and chemical data. South Atlantic coast of the United States. Theodore N. Gill Cruise 3. Spec. Sci. Rep., U. S. Fish. Wildl. Serv., 210: 1-208.
Backus, Richard H.
1957. Notes on western North Atlantic sharks. Copeia, 1957 (3): 246-248.
Barcellos, B. N.
1957. Ictiofauna do Rio Grande do Sul. I. Sobre *Heptranchias perol* (Bonnaterre, 1788). Rev. Brasil. Biol., *17*(2): 173-178.

Böhlke, James E. and Charles C. G. Chaplin
1957. Oral incubation in Bahamian jawfishes, *Opisthognathus whitehursti* and *O. maxillosus*. Science, *125*(3243): 353.

Böhlke, James E.
1957. The Bahaman species of emblemariid blennies. Proc. Acad. nat. Sci. Philad., *109*: 25-57, 3 pls.
1957. On the occurrence of garden eels in the Western Atlantic, with a synopsis of the Heterocongrinae. Proc. Acad. nat. Sci. Philad., *109*: 59-79, pl. 4.
1957. A review of the blenny genus *Chaenopsis*, and the description of a related new genus from the Bahamas. Proc. Acad. nat. Sci. Philad., *109*: 81-103, pls. 5-6.
1957. Notes on the serranid fish *Chorististium rubrum* (Poey) and on the status of *Ypsigramma* Schultz. Not. Nat. Philad., 291: 1-7, 1 pl.
1957. A new shallow-water brotulid fish from the Great Bahama Bank. Not. Nat., Philad., 295: 1-8.

Briggs, John C. and David K. Caldwell
1957. *Acanthurus randalli*, a new surgeon fish from the Gulf of Mexico. Bull. Fla. State Mus., *2*(4): 43-51.

Caldwell, David K.
1957. The biology and systematics of the pinfish, *Lagodon rhomboides* (Linnaeus). Bull. Fla. State Mus., *2*(6): 77-173.

DeSylva, Donald P.
1957. Studies on the age and growth of the Atlantic sailfish, *Istiophorus americanus* (Cuvier), using length frequency curves. Bull. Mar. Sci. Gulf & Carib., *7*(1): 1-20.

Ege, Vilh
1957. Paralepididae II. (*Macroparalepis*). Taxonomy, ontogeny, phylogeny distribution. Dana Rep. 43: 1-101.

Hubbs, Clark
1957. *Gambusia heterocheir*, a new poeciliid fish from Texas, with an account of its hybridization with G. *affinis*. Tulane Stud. Zool., *5*(1): 1-16.

Hubbs, Clark and Victor G. Springer
1957. A revision of the *Gambusia nobilis* species group, with descriptions of three new species, and notes on their variation, ecology, and evolution. Texas J. Sci., *9*(3): 279-327.

Kanazawa, Robert H.
1957. A new species of eel, *Coloconger meadi*, and new records for the ateliopid fish, *Ijimaia antillarum*. Howell Rivero, both from the Gulf of Mexico. Copeia, 1957 (3): 234-235.

Martin S., Felipe
1957. Una nueva especie de Torpedinidae del Golfo de Coriaco, Edw. Sucre, Venezuela. Novedades cientif. Contrib. Ocass. Mus. Hist. nat. La Salle, 21: 1-4.

Maul, G. E.
1957. Further additions to the previously revised family Searsidae. Bol. Mus. Mun. Funchal, *10:* 3-21.

Mead, Giles W.
1957. On the bramid fishes of the Gulf of Mexico. Zoologica, N. Y., *42*(4): 51-61, 3 pls.

Miller, Robert Rush
1956. A new genus and species of cyprinodontid fish from San Luis Potosi, Mexico, with remarks on the subfamily Cyprinodontinae. Occ. Pap. Mus. Zool., Michigan, No. 581: 1-17, 2 pls.

Morrow, James E.
1957. A redefinition of the subspecies of *Fodiator acutus*. Postilla, *29:* 1-10.

Nybelin, Orvar
1957. Deep-sea bottom-fishes. Rep. Swedish Deep-Sea Exped., *2,* (Zool.) (20): 247-345, 7 pls.

Reid, George K.
1957. External morphology of an embryo whale shark, *Rhineodon typus* Smith. Copeia, 1957(2): 157-158.

Schultz, Leonard P.
1957. The frogfishes of the family Antennariidae. Proc. U. S. nat. Mus., *107*(3383): 47-105, 14 pls.

Shoemaker, Hurst H.
1957. Observations of bioluminescence in the Atlantic fish *(Porichthys porosissimum).* Science, *126*(3283): 1112.

Smith, C. Lavett
1957. Two new clinid blennies *(Malacoctenus)* from Puerto Rico. Occ. Pap. Mus. Zool., Univ. Michigan, 585: 1-15, 1 pl.

Springer, Stewart and John R. Thompson
1957. Night sharks, *Hypoprion,* from the Gulf of Mexico and the Straits of Florida. Copeia, 1957(2): 160.

Springer, Victor G.
1957. A new genus and species of elopid fish *(Laminospondylus transversus)* from the Upper Cretaceous of Texas. Copeia, 1957(2): 135-140, 1 pl.

Tucker, Denys W.
1957. Studies on the trichiuroid fishes. IV. A specimen of *Evoxymetopon taeniatus* (Poey) Gill, from the Gulf of Mexico. Ann. Mag. nat. Hist., *10*(114): 425-428.

Voss, Nancy A.
1957. Fishes of the family Gempylidae collected by the Bermuda Oceanographic Expeditions 1929, 1930, 1931 and 1934. Copeia, 1957(4): 304-305.

Walters, Vladimir
1957. A new sea bass from the West Indies. Copeia, 1957(4): 283-286.

Ward, J. W.
1957. The reproduction and early development of the sea catfish, *Galeichthys felis,* in the Biloxi (Mississippi) Bay. Copeia, 1957(4): 295-298.

Weibezahn, Franz H.
1952. Contribution al conocimiento de los clupeidos de Venezuela. Bol. Pesca, Caracas, *3:* 1-21, 2 pls.

Winn, E. Howard and J. E. Bardach
1957. Behavior, sexual dichromatism, and species of parrot fishes. Science, *125*(3253): 885-886.

Woolcott, William S.
1957. Comparative osteology of serranid fishes of the genus *Roccus* (Mitchill). Copeia, 1957(1): 1-10, 2 pls.

Distribution and Ecology

Backus, Richard H.
1957. Northern record of the snake eel *Ophichthus gomesi* (Castlenau). Copia, 1951(1): 61.

Berry, Frederick H.
1957. Addition to the fishes known from the vicinity of Cedar Keys, Florida. Quart. J. Fla. Acad. Sci., *20*(4): 232.

Breuer, Joseph P.
1957. An ecological survey of Baffin and Alazan Bays, Texas. Publ. Inst. Mar. Sci., *4*(2)L 134-155.

Caldwell, David K.
1957. Additional records of marine fishes from the vicinity of Cedar Key, Florida. Quart. J. Fla. Acad. Sci., *20*(2): 126-128.

Caldwell, David K. and John C. Briggs
1957. Range extensions of western North Atlantic fishes with notes on some soles of the genus *Gymnachirus*. Bull. Fla. State Mus., *2*(1): 1-11.

Duarte-Bello, Pedro P. and Jose A. Suarez Caabro
1957. *Chriodorus atherinoides* nuevo reporte para la fauna ictiologica cubana. Univ. Catolica de Sto. Tomas de Villanueva, Bol. 2: 1-3.

Gibbs, Robert H., Jr.
1957. Preliminary analysis of the distribution of white marlin, *Makaira albida* (Poey), in the Gulf of Mexico. Bull. Mar. Sci. Gulf & Carib., *7*(4): 360-369.

Gunter, Gordon
1957. Predominance of the young among marine fishes found in fresh water. Copeia, 1957(1): 13-16.

Joseph, Edwin B. and Ralph W. Yerger
1956. The fishes of Alligator Harbor, Florida, with notes on their natural history. Pap. Oceanogr. Inst. Fla. State Univ., 2: 111-156, 4 pls.

Kemp, Robert J., Jr.
1957. Occurrences of the ocean sunfish, *Mola mola* (Linnaeus) in Texas. Copeia, 1957(3): 250-251.

Krumholz, Louis B.
1957. A record of the night shark, *Hypoprion signatus* from Bimini, Bahamas. Copeia, 1957(4): 300-301.

Leary, Terrance R.
1957. The bonefish, *Albula vulpes*, in Texas. Copeia, 1957(3): 248-249.

Mead, Giles W.
1957. An Atlantic record of the zeoid fish *Parazen pacificus*. Copeia, 1957(3): 235-237, 1 pl.

Pew, Patricia
1957. Occurrence of young dolphin, *Coryphaena hippurus*, in a Texas Bay. Copeia, 1957(4): 300.

Robins, C. Richard
1957. Effects of storms on the shallow-water fish fauna of southern Florida with new records of fishes from Florida. Bull. Mar. Sci. Gulf & Carib., *7*(3): 266-275.

Simmons, Ernest G.
1957. An ecological survey of the upper Laguna Madre of Texas. Publ. Inst. Mar. Sci., *4*(2): 156-200.

Simpson, Don G. and Gordon Gunter
1956. Notes on the habitats, systematic characters and life histories of Texas saltwater cyprinodontes. Tulane Stud. Zool., *4*(4): 115-134.
Springer, Stewart
1957. Some observations on the behavior of schools of fishes in the Gulf of Mexico and adjacent waters. Ecology, *39*(1): 166-171.
Suttkus, Royal D.
1956. First record of the mountain mullet, *Agonostomus monticola* (Bancroft) in Louisiana. Proc. La. Acad. Sci., *19:* 43-46.
Wodd, F. G., Jr.
1957. Southern extension of the known range of the basking shark, *Cetorhinus maximus* (Gunnerus). Copeia, 1957 (2): 153-154.
Woods, Loren P.
1957. *Beryx splendens* Lowe in the Gulf of Mexico. Copeia, 1957(4): 298-299.

Marine Fisheries

General

Anderson, A. W.
1957. Fish consumption—where is it headed? Proc. Gulf & Carib. Fish. Inst., 9th Ann. Sess. 1956: 88-92.
Bayitch, S. A.
1957. International law of fisheries. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 33-39.
Farley, John L.
1957. The new place of commercial fisheries in the United States Government. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 93-98.
Marshall, Arthur R.
1957. The activities of the U. S. Fish and Wildlife Service, Office of River Basin Studies, in relation to federal water-use projects and the marine fishery resources of Florida. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 175-180.
Rawls, Oscar
1957. Activities of the Corps of Engineers related to Florida coastal fisheries. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 171-175.

Fisheries Technology

Camber, C. Isaac, James E. Alexander and Mary H. Vance
1957. The effect of the antibiotic chlortetracycline on shrimp freshness. (Abstract). Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 32.
Camber, C. Isaac, Mary H. Vance and James E. Alexander
1957. The use of sodium bisulfite for the control of black spot in shrimp. Fla. State Bd. Conserv. Tech Ser. 20:
Farber, Lionel and Peter A. Leske
1957. The effect of chlortetracycline on shrimp spoilage at various temperatures. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 26-32.
Fellus, C. R., M. Gagnon and R. Khatchkian
1957. Biochemical and bacteriological methods for determining shrimp quality. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 23-26.
Gardner, Elizabeth Ann and Betty M. Watts
1957. Deterioration of cooked southern oysters. Food. Technol., *11:* 6-11.

Gardner, Elizabeth Ann and Betty M. Watts
1957. Effect of ionizing radiations on southern oysters. Food Techn., *11:* 329-331.
Miller, Theodore M.
1957. Progress in technological studies on Menhaden products under the Saltonstall-Kennedy program. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 48-54.
Olcott, Harold S.
1957. Oxidation deterioration in fish and fishery products. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 55-56.
Schwartz, Mark G. and Betty M. Watts
1957. Application of the thiobarbituric acid test as a quantitative measure of deterioration in cooked oysters. Food Res., *22:* 76-81.
Westerlund, Barton A., James Murdock, Jack T. Brawner, C. P. Idyll and Albert Rosen
1957. A summary of the studies on the marketing of Florida fish. (Abstract). Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 87.

Fishing Vessels and Gear

Danforth, Warner E. and Chris A. Theodore
1957. Some findings on insurance experience of commercial fishing vessels in the Gulf of Mexico. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 64-78.

Marine Fishery Investigation

General

Bardach, John E. and David W. Menzel
1957. Field and laboratory observation on the growth of some Bermuda fishes. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 106-112.
Butler, Charles
1957. A new federal fishery laboratory on the Gulf. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 45-47.
Carranza, Jorge
1957. Marine fisheries of the Yucatan Peninsula, Mexico. Proc. Gulf & Carib. Fish Inst. 9th Ann. Sess. 1956: 145-150.
June, Fred C.
1957. Biological investigations of Atlantic coast Menhaden. Proc. Gulf & Carib. Fish Inst. 9th Ann. Sess. 1956: 99-106.
Murdock, James
1957. A survey of spearfishing in the Florida Keys. Proc. Gulf & Carib. Fish Inst. 9th Ann. Sess. 1956: 112-120.
Regan, James, C. P. Idyll and Edwin S. Iversen
1957. Mesh size regulation as a possible method of managing the Tortugas shrimp fishery. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 18-22.
Rosa, H. Jr.
1957. The survey of living aquatic resources. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 153-170.
Springer, Stewart
1957. Some observations on the behavior of schools of fishes in the Gulf of Mexico and adjacent waters. Ecology, *38* (1): 166-171.

Springer, Stewart
1957. Tuna resources of the tropical and subtropical Western Atlantic. Trans. Amer. Fish. Soc., *85* (1955): 13-17.

Stein, Jerome E.
1957. The status of scientific knowledge about the red snapper (*Lutjanus aya* Bloch) and the economic importance of the fishery. (Abstract). Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 99.

Storr, John F.
1957. Progress of recovery of the commercial sponge beds of Florida. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 120-127.

Storr, John F.
1957. The sponge industry of Florida. Fla. State Bd. Conserv. Ed. Series 9: 1-29.

Suarez Caabro, Jose A.
1957. The present situation in the fishing industry in Cuba. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 136-145.

Soumela, Arnie J.
1957. The Federal Government's role in the development of new fishery resources. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 1-4.

Tabb, Durbin
1957. Studies on the life history of the spotted sea trout, *Cynoscion nebulosus* (C. and V.). (Abstract). Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess., 1956: 106.

Wiles, D. W.
1957. Developing a Caribbean fishery. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 150-153.

Shell Fisheries

Crustacea

Bullis, Harvey R., Jr.
1957. Royal red shrimp—a new South Atlantic resource. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 56-60.

Costello, Thomas J.
1957. Aims and progress in Gulf Fishery Investigation's shrimp research. Proc. Gulf & Carib. Fish, Inst. 9th Ann. Sess. 1956: 5-6.

Idyll, Clarence P.
1957. The commercial shrimp industry of Florida. Fla. State Bd. Conserv., Ed. Ser. 6.

Ingle, Robert M.
1957. Intermittent shrimp sampling in Apalachicola Bay with biological notes and regulating application. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 6-17.

Kahn, Richard A.
1957. Ways and means of stabilizing the shrimp market. Proc. Gulf & Carib. Fish. Inst. 9th Ann. Sess. 1956: 78-87.

Woodburn, K. D., B. Eldred, E. Clark, R. H. Hutton, and R. M. Ingle
1957. The live bait shrimp industry of the west coast of Florida (Cedar Keys, to Naples). Fla. State Bd. Conserv. Tech Series 21: 1-33.

YOUNG, ROBERT
1957. Florida crab plant design and sanitation. Fla. St. Bd. Conserv., Ed. Series 10: 1-20.

Mollusks

FINGERMAN, MILTON, LAURENCE D. FAIRBANKS and WARREN C. PLAUCHE
1957. Body fluid losses of northern and southern oysters. Comm. Fish. Rev., *19*(9): 16-18.